

Association of non-alcoholic fatty liver disease and fibrosis with incident dementia and cognitive function: The Rotterdam Study

Tian Xiao^{a,1}, MD, Laurens A. van Kleef^{b,1}, MD, M. Kamran Ikram^{a,c}, PhD, Robert J. de Knegt^{b,*}, PhD, and M. Arfan Ikram^{a,*}, PhD

^aDept of Epidemiology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands.

^bDept of Gastroenterology and Hepatology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands.

^cDept of Neurology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands.

¹Authors contributed equally

Supplementary files

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eTable 1: details of the cognitive tests used in cross-sectional analysis

Letter Digit Substitution Task (LDST)	Processing speed was measured with the LDST. After providing an example with the correct combinations, participants were asked to make as many letter-digit combinations in 60 seconds as possible.
Stroop tests	Attention and concentration were measured using the Stroop test, which comprises three trials. In the first trial, participants are asked to name the printed word of a card which contains color names in black. In the second trial, participants were asked to name the printed color of colored blocks. And last, in the third trial, participants were asked to name the color of the ink of the card that contains color names printed in different ink colors than the actual color name.
Word Fluency Task (WFT)	Verbal fluency was measured with the WFT. Participants were asked to provide as many animal names as possible during 60 seconds.
15-Verbal word learning test (WLT)	Immediate recall and delayed recall were measured with the WLT. First, participants were provided a list of 15 unrelated words and were asked to repeat them after five different trials and receiving another list of 15 unrelated words (WLTimm). This was asked again after 30 minutes (WLTdel).
Purdue Pegboard test (PBB test)	Unilateral and bilateral fine manual dexterity was quantified using the PPB. This test uses a pegboard comprising two rows, with 25 holes each and a number of pins. Participants get 30 seconds to place as many pins as possible in the holes in the prescribed order. First with the right hand, then the left and last using both hands.

eTable 2: Application of exclusion criteria per set.

	Set 1	Set 2	Set 3
Eligible for inclusion	6.048	5.967	4.540
Dementia at baseline	79	45	27
No dementia data	4	10	7
No follow-up	24	22	17
NAFLD exclusion criteria			
Viral hepatitis	25	43	34
Excessive alcohol*	1.430	776	629
Steatogenic drug use	115	97	68
Missing alcohol data	396	397	315
Invalid liver stiffness [†]	-	-	143
Total participants excluded	2.073	1.390	1.240
Participants included	3.975	4.577	3.300

*Excessive alcohol: daily intake of >30 grams for male and > 20 grams for female. [†]According to the Boursier criteria.

Abbreviations: NAFLD, non-alcoholic fatty liver disease.

eTable 3: baseline characteristics by NAFLD status for incident dementia analysis

	Set 1: Fatty liver index			Set 2: Ultrasound	
	FLI < 30 n = 1335	FLI 30 – 60 n = 1347	FLI ≥ 60 n = 1293	No NAFLD n = 2991	NAFLD n = 1586
Demographics					
Age (years)	70.2 (8.3)	70.3 (8.0)	69.6 (7.7)	69.9 (9.5)	69.9 (8.5)
Female	949 (71.1)	745 (55.3)	714 (55.2)	1783 (59.6)	926 (58.4)
Alcohol consumption	1023 (76.6)	1067 (79.2)	978 (75.6)	2523 (84.4)	1343 (84.7)
Former/current smoking	749 (56.5)	873 (65.3)	873 (67.7)	1885 (62.1)	1078 (68.1)
Educational level					
Low	804 (60.7)	780 (58.3)	773 (60.4)	1379 (46.6)	858 (54.8)
Intermediate	359 (27.1)	400 (29.9)	370 (28.9)	911 (30.8)	444 (28.4)
High	162 (12.2)	158 (11.8)	136 (10.6)	671 (22.7)	263 (16.8)
Physical examination					
BMI (kg/m ²)	23.5 (2.3)	26.8 (2.3)	30.9 (3.7)	26.2 (3.7)	30.2 (4.4)
Enlarged waist circumference*	121 (9.1)	601 (44.6)	1077 (83.3)	895 (29.9)	1120 (70.6)
Comorbidity					
Metabolic syndrome	230 (17.3)	670 (49.9)	1083 (84.0)	1159 (39.4)	1109 (71.2)
Diabetes	73 (5.5)	157 (11.7)	319 (24.7)	313 (10.6)	402 (25.8)
Stroke	20 (1.5)	22 (1.6)	29 (2.2)	82 (2.7)	40 (2.5)
Hypertension	759 (56.9)	928 (69.0)	1040 (80.7)	2061 (68.9)	1313 (82.8)
Biochemistry / genetics					
ALT (U/L)	18 [15, 21]	20 [16, 25]	23 [18, 30]	17 [14, 22]	21 [16, 28]
GGT (U/L)	17 [14, 23]	23 [17, 30]	31 [23, 44]	21 [15, 30]	28 [20, 39]
Cholesterol (mmol/L)	5.72 (0.95)	5.83 (0.98)	5.80 (0.99)	5.46 (1.10)	5.35 (1.12)
Triglycerides (mmol/L)	1.04 [0.84, 1.30]	1.37 [1.08, 1.79]	1.80 [1.39, 2.38]	1.16 [0.91, 1.53]	1.56 [1.18, 2.07]
APOE-ε4	374 (29.2)	357 (27.4)	331 (26.7)	809 (29.0)	328 (22.3)

Data is presented as mean (SD), median [P25-P75] or n and percentage. Baseline characteristics are presented for set 1 and set 2, stratified for NAFLD status. *Waist circumference > 102 cm for male and > 88 cm for female.

Abbreviations: APOE, apolipoprotein E; ALT, alanine transaminase; BMI, body mass index; FLI, fatty liver index; GGT, gamma glutamyl transpeptidase; NAFLD, non-alcoholic fatty liver disease.

eTable 4: baseline characteristics by fibrosis status for incident dementia analysis

	Set 3: Fibrosis	
	LSM < 8.0 kPa n = 3108	LSM ≥ 8.0 kPa n = 192
Demographics		
Age (years)	67.4 (8.3)	71.2 (9.3)
Female	1820 (58.6)	72 (37.5)
Alcohol consumption	2669 (85.9)	161 (83.9)
Former/current smoking	1941 (62.6)	140 (72.9)
Educational level		
Low	1436 (46.7)	81 (42.2)
Intermediate	907 (29.5)	65 (33.9)
High	733 (23.8)	46 (24.0)
Physical examination		
BMI (kg/m ²)	27.0 (3.8)	28.8 (4.6)
Enlarged waist circumference*	1246 (40.1)	110 (57.3)
Comorbidity		
Metabolic syndrome	1395 (45.6)	134 (70.5)
Diabetes	395 (12.8)	63 (33.0)
Stroke	57 (1.8)	2 (1.0)
Hypertension	2105 (67.7)	171 (89.1)
Biochemistry / genetics		
ALT (U/L)	18 [14, 23]	23 [17, 33]
GGT (U/L)	22 [16, 32]	34 [23, 64]
Cholesterol (mmol/L)	5.50 (1.10)	5.07 (1.08)
Triglycerides (mmol/L)	1.26 [0.96, 1.69]	1.31 [1.00, 1.87]
APOE-ε4	799 (27.7)	43 (23.6)

Data is presented as mean (SD), median [P25-P75] or n and percentage. Baseline characteristics are presented for set 3, stratified for fibrosis status. *Waist circumference > 102 cm for male and > 88 cm for female. Abbreviations: APOE, apolipoprotein E; ALT, alanine transaminase; BMI, body mass index; FLI, fatty liver index; GGT, gamma glutamyl transpeptidase; NAFLD, non-alcoholic fatty liver disease.

eTable 5: Mean difference of performance on cognitive tests between participants with NAFLD compared to no NAFLD and fibrosis compared to no fibrosis expressed in z-scores.

Cognitive test	NAFLD			Fibrosis		
	n	MD	95% CI	n	MD	95% CI
G-factor, z-score	3574	0.032	-0.029; 0.092	2657	-0.172	-0.307; -0.037*
LDST, z-score	4414	0.042	-0.019; 0.103	3197	-0.201	-0.335; -0.067*
Stroop test1*, z-score	4425	-0.047	-0.114; 0.021	3204	0.199	0.053; 0.345*
Stroop test2*, z-score	4424	-0.079	-0.148; -0.011*	3203	0.135	-0.015; 0.284
Stroop test3*, z-score	4415	-0.046	-0.110; 0.019	3199	0.179	0.041; 0.316*
WFT, z-score	4503	0.000	-0.065; 0.065	3248	-0.132	-0.274; 0.010
WLTdel, z-score	4193	-0.027	-0.093; 0.039	3028	-0.024	-0.165; 0.118
WLTimm, z-score	4193	-0.011	-0.076; 0.054	3027	-0.006	-0.145; 0.133
WLTrecog, z-score	4290	-0.012	-0.082; 0.058	3088	-0.054	-0.203; 0.096
PPB test, z-score	3982	0.051	-0.009; 0.111	2958	-0.086	-0.219; 0.048

Higher scores indicate better cognitive function, except for Stroop tests. Results were obtained from linear regression analyses and Tukey all-pair comparisons method based on ANOVA models. Differences were calculated for the individual cognitive tests and G-factor for participants with NAFLD compared to those without NAFLD and for fibrosis compared to no fibrosis. Results were adjusted for age, sex, education level, smoking status, BMI, cholesterol, triglycerides, hypertension, stroke, diabetes, depression and APOE genotypes.

Abbreviations: *APOE*, apolipoprotein E; CI, confidence interval; G-factor, principle component scores of cognition tests; LDST, Letter-Digit Substitution test; MD, Mean difference; NAFLD, non-alcoholic liver disease; PPB test, Purdue Pegboard test; WFT, Word Fluency test; WLTdel, Word learning test, delayed recall; WLTimm, Word learning test, immediate recall; WLTrecog, Word learning test, recognition.