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CLINICAL STUDIES

Heritability of non-alcoholic fatty liver disease and association with abnormal vascular parameters: A twin study

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) has been linked to increased cardiovascular morbidity. However, genetic factors have an unclear role in this condition. *Aims:* To analyse heritability of NAFLD and its association with abnormal vascular parameters in a large twin cohort. *Methods:* Anthropometric and lipid metabolic parameters were obtained from 208 adult Hungarian twins (63 monozygotic and 41 dizygotic pairs; 58 men and 150 women; age 43.7 ± 16.7 years). B-mode ultrasonography was performed to detect steatosis and categorize severity. Brachial and aortic augmentation indices and aortic pulse wave velocity were assessed using oscillometry (TensioMed Arteriograph). Carotid intima media thickness (IMT) was measured using ultrasonography on the proximal common, distal common and internal carotid arteries. *Results:* NAFLD was identified in 47 subjects (22.6%), of which 44 (93.6%) had mild and 3 (6.4%) had moderate steatosis. These subjects were older (age: 50.9 ± 14.3 vs. 41.5 ± 16.7 years, $P < 0.001$) and had a higher body mass index (BMI; 30.1 ± 5.2 vs. 24.6 ± 4.1 km/m^2, $P < 0.001$) than non-NAFLD twins. Based on 91 same-sex twin pairs, heritability analysis indicated no discernible role for genetic components in the presence of NAFLD (95% confidence interval, 0.0–36.0%), while shared and unshared environmental effects accounted for 74.2% and 25.8% of variations adjusted for age and BMI. Augmentation indices and carotid IMT in twins with NAFLD were increased at most examined locations ($P < 0.05$–$P < 0.001$).*Conclusion:* These findings do not support heritability of NAFLD, although it coexists with vascular parameters linked to increased cardiovascular risk, underscoring the importance and value of prevention in this very common disorder.

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver disorders that include isolated steatosis, characterized by ectopic fat accumulation in the liver, and non-alcoholic steatohepatitis, characterized by steatosis combined with additional features such as hepatocellular injury, inflammation and fibrosis (1). Depending on severity of the disease, NAFLD may predictably progress into cirrhosis and hepatocellular carcinoma (1, 2). Although the overall risk for this progression is relatively low, NAFLD may become a major cause of liver-related morbidity and mortality in both adults and children because of an unusually high prevalence in the developed world (1).

NAFLD is often regarded as the hepatic manifestation of the metabolic syndrome, which is a variable constellation of obesity, diabetes, hyperlipidaemia, and hypertension (1, 3). Accordingly, NAFLD has been linked to an adverse cardiovascular risk profile both in adults and in the paediatric population (4, 5). Recent studies indicate

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that the presence of NAFLD is associated with diminished coronary flow reserve, increased likelihood of calcified and non-calcified coronary plaque formation, and atherosclerosis in the internal carotid artery (5, 6). Structural and functional vascular parameters predicting the risk of cardiovascular events also include increased carotid intima media thickness (IMT), arterial stiffness (PWV, pulse wave velocity) and impaired endothelial function, which were found to be independent predictors of the corresponding risk (7, 8). NAFLD has been associated with many of these vascular parameters (9).

The relative contribution of genetic and environmental factors that determine the natural history of NAFLD and its association with cardiovascular disease is not clear. The role of heredity in developing NAFLD was suspected in some family studies with a possible genetic risk (10) and familiar clustering of 18% (11). NAFLD has a two-fold higher prevalence among Hispanics compared to African Americans, and a recent study found that the heritability of NAFLD reached 35% in a Hispanic American cohort (12). A family study conducted among the relatives of overweight children with biopsy-proven NAFLD reported an overall high heritability of fatty liver and a moderate heritability of liver fat fraction as determined using MR spectroscopy (13). A twin study investigated the genetic covariance between gamma-glutamyl transpeptidase and fatty liver risk factors, and reported linked pathways between genetic susceptibility to NAFLD and the metabolic syndrome (14).

To our knowledge, no study has been performed among healthy adult twins to investigate the role of heredity in NAFLD and its association with cardiovascular disease. Our goal was, therefore, to study the heritability of NAFLD and its relationship with structural and functional vascular parameters linked to increased cardiovascular risk in a large twin cohort. To this end, we estimated the influence of genetic as well as shared and unshared environmental components not applicable in previously performed family design studies. We hypothesized that NAFLD would have low or moderately low heritability in line with the findings of prior non-twin studies. Since NAFLD is closely linked to metabolic syndrome, we also assumed that there would be a strong association between the presence of NAFLD and abnormal vascular parameters.

Methods

Subjects and study design

A total of 208 twin subjects over the age of 18 (104 healthy Hungarian twin pairs; 63 monozygotic and 41 same- and opposite-sex dizygotic pairs; 58 men and 150 women) were tested in this cross-sectional twin study. Exclusion criteria included significant use of alcohol (men: greater than 20 g/day; women: greater than 10 g/day), pregnancy and foreseeable lack of compliance with test procedures. In the absence of genotyping and to maximize the accuracy of zygosity classification, we used a multiple-choice self-reported questionnaire. Zygosity was assigned according to a seven-part self-reported response (15). All study subjects gave informed consent prior to entering the study, which was conducted in full compliance with regulations of the Ethical Committee of Semmelweis University. For power calculations, we assumed 10% difference (effect size) between two groups (e.g., NAFLD vs. non-NAFLD) with 20% standard deviation, 5% two-sided alpha risk, and 80% power, which yielded a minimum group size of 32 indicating sufficient statistical power for critical comparisons.

Anthropometric and vascular measurements were obtained at two twin festivals in Hungary (Agfalva and Szigethalom). Participants were asked to complete a questionnaire to identify clinical symptoms and to obtain complete past medical history and a list of medications for cardiovascular or liver conditions. Imaging studies were subsequently performed at two large hospitals in Budapest, Hungary (Department of Radiology and Oncotherapy, Semmelweis University, and Department of Cardiology, Military Hospital - National Health Center). In addition, study subjects underwent phlebotomy in these hospitals to determine the following laboratory parameters: serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride and C reactive protein (CRP). Prior to phlebotomy, subjects were restricted from eating for at least 1 h, and from drinking alcohol or coffee for 10 h to avoid the effect of food consumption on serum levels of triglycerides and cholesterol.

Fatty liver assessment

Limited abdominal sonography was performed by using B-mode ultrasonography (Esaote MyLab 70X Vision, Genova, Italy) equipped with a curved array transducer (1–8 MHz, CA431). Standardized digital images of the liver and right kidney were recorded and examined by a specialized radiologist blinded to the subjects' twinship and clinical characteristics. The extent of fatty liver was determined by following standard technique as described earlier (16, 17). Accordingly, steatosis was based on increased echogenicity, loss of portal vein walls, decreased through-transmission and closely packed echoes categorized into four groups: (i) normal liver without evidence for increased fat content and characterized by normal hepatic echogenicity and normal beam attenuation; (ii) mild steatosis, defined as the slight increase in echogenicity of liver parenchyma compared with the right kidney parenchyma and showing minimal or no decrease of visualization of hepatic vessels or their walls and the diaphragm; (iii) moderate steatosis, characterized by the diffuse increase in liver echogenicity and slightly impaired visualization of intrahepatic vessels or their walls and the diaphragm; and (iv) severe steatosis, defined as marked increase of liver

echogenicity, poor visualization of intrahepatic vessels or their walls and increased posterior beam attenuation represented by non-visualization of the diaphragm.

Anthropometric data

Body mass index (BMI) measurements were carried out by a clinically validated OMRON BF500 body consistency monitor (Omron Healthcare Ltd., Kyoto, Japan). Current height was verified simultaneously.

Arterial stiffness measurements

Brachial and aortic augmentation indices (AIx) and aortic pulse wave velocity (PWV_{ao}) were assessed by noninvasive oscillometry using TensioMed Arteriograph (1.10.1.1. software) (18, 19). Study subjects assumed the supine position to decrease inter- and intra-observer variability and in accordance with guidelines recommended by the European Society of Cardiology (20). If automatic quality control was appropriate at first (standard deviation for PWV_{ao} less than 1.0), only one measurement was performed, otherwise the subject underwent at least three measurements. All subjects were restricted from smoking for 3 h, from eating for 1 h, and from drinking alcohol or coffee for 10 h prior to measurements.

Carotid intima media thickness measurements

Carotid ultrasonography was carried out via high-resolution B-mode ultrasonography (Toshiba Power Vision, Tokyo, Japan and Esaote MyLab 70X Vision) by using linear array transducers (5–10 MHz, LA523). Intima media thickness (IMT) of the proximal and distal common carotid arteries (CCAs) and the proximal internal carotid artery (ICA) were measured bilaterally with standard techniques (21, 22). IMT was quantified on the far wall of the CCA at 3-5 cm after its origin from the subclavian artery (proximal CCA) and at 1 cm proximal to the bifurcation (distal CCA). In addition, IMT was measured on the far wall at the proximal left and right ICA at 1 cm distal to the bifurcation. For each segment, the sonographer used multiple different scanning angles to identify the longitudinal image of IMT showing the maximum IMT. Average values of the IMT of each of the six measurement spots (right and left proximal CCA, distal CCA, proximal ICA) were used to compute the IMT values for each twin in the analysis. No electrocardiogram gating was applied during these measurements. In case of a carotid plaque, IMT was determined below the end of the plaque.

Statistical analysis

A descriptive analysis (mean, standard deviation and percentage for categorical variables) for fatty liver, anthropometric, laboratory values, and vascular parameters was conducted by SPSS Statistics 17. P values less than 0.05 were considered significant. Heritability estimates were determined based on the consideration that greater levels of monozygotic than dizygotic within pair similarity indicate a genetic influence on a phenotype, while similarity of co-twin correlations suggests that the variance is because of shared environmental sources. Structural equation modelling was performed by using the Mplus Version 6.1 weighted least squares estimation caused by the categorical variable of interest (23). Empirical confidence intervals were calculated with a Bollen-Stine Bootstrap (24). Univariate quantitative genetic modelling was performed to decompose the phenotypic variance of the considered parameters into heritability (A), shared (C) and unshared (E) environmental effects (ACE analysis). The additive genetic component (A) measures the effects caused by genes at multiple loci or multiple alleles at one locus. The shared environmental component (C) estimates contribution of a common family environment for both twins (e.g., familiar socialization), whereas the unshared environmental component (E) estimates the effects that separately apply to each individual twin and accounts for measurement errors.

Results

Prevalence of NAFLD in twins

Baseline characteristics of all study subjects and the sub-group of same-sex monozygotic and dizygotic twins according to zygosity are presented in Table 1. To assess heritability of sonographically determined NAFLD in our cohort, we excluded opposite-sex dizygotic twin pairs from the ACE model. Regardless of zygosity, female gender dominated in both groups. Dizygotic twins were significantly older ($P < 0.05$). No significant difference was observed in anthropometric parameters between monozygotic and dizygotic twins except height ($P < 0.05$). However, this difference did not translate into different BMIs between monozygotic and dizygotic twins, indicating a similar degree of overweight in both groups (26.0 ± 5.1 kg/m^2 vs. 25.9 ± 4.9 kg/m^2, NS). Similarly, routine parameters of lipid metabolism or the serum level of CRP showed no significant differences according to zygosity except higher LDL-cholesterol levels in the sub-group of 27 same-sex dizygotic twin pairs when compared with monozygotic twins ($P < 0.05$). Interestingly, prevalence of familial combined hyperlipidaemia was higher in dizygotic twins ($P < 0.01$). Subjects were classified as having familial combined hyperlipidaemia when total cholesterol and/or triglyceride levels exceeded the 90th percentile adjusted for age and gender.

The degree of liver steatosis was detected by ultrasonography. The sonographic presence of fatty liver was considered indicative of NAFLD in the absence of chronic liver disease and significant alcohol consump-

Table 1. Baseline characteristics of study subjects

	Total (n = 208)*	Same-sex (n = 182)†	Monozygotic (n = 128)†	Dizygotic (n = 54)†	P-value
Male: female	59 : 149	46: 136	30 : 98	16 : 38	NS
Age, years	43.7 ± 16.7	44.5 ± 16.8	42.8 ± 16.8	48.5 ± 16.1	<0.05
Weight, kg	72.6 ± 15.1	72.0 ± 15.3	71.0 ± 15.2	74.5 ± 15.3	NS
Height, cm	167.3 ± 10.1	166.5 ± 9.8	165.2 ± 9.7	169.3 ± 9.9	<0.05
BMI, kg/m^2	25.8 ± 5.0	25.9 ± 5.0	26.0 ± 5.1	25.9 ± 4.9	NS
Total cholesterol, mmol/L	5.1 ± 0.9	5.1 ± 0.9	5.0 ± 0.9	5.3 ± 0.9	NS
HDL cholesterol, mmol/L	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	NS
LDL cholesterol, mmol/L	3.2 ± 0.9	3.2 ± 0.9	3.1 ± 0.8	3.5 ± 0.9	<0.05
Triglycerides, mmol/L	1.5 ± 0.9	1.5 ± 0.9	1.5 ± 0.9	1.5 ± 0.9	NS
CRP, mg/L	2.2 ± 2.8	2.2 ± 2.7	2.1 ± 2.7	2.5 ± 2.8	NS
Fatty liver, n (%)	47 (22.6)	47 (25.8)	33 (25.8)	14 (25.9)	NS
Mild	44 (21.4)	44 (24.2)	30 (23.4)	14 (25.9)	NS
Moderate	3 (1.4)	3 (1.6)	3 (2.3)	0 (0)	NS
Severe	0 (0)	0 (0)	0 (0)	0 (0)	NS
Familial combined hyperlipidaemia‡, n (%)	31 (14.9)	27 (14.8)	18 (14.1)	13 (24.1)	<0.01

*104 twin pairs including opposite-sex twin pairs;

†monozygotic and same-sex dizygotic twin pairs were included in the heritability analysis;

‡Characterized by a variable expression of hypercholesterolaemia and hypertriglyceridaemia.

BMI, body mass index; CRP, C reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant. Data are shown as mean ± standard deviation where appropriate.

tion in the past medical history. Accordingly, the overall prevalence of sonographically detected NAFLD in our twin cohort was 22.6%, which is similar to estimates made on the general population of industrialized countries. Based on sonographic criteria, the degree of steatosis was categorized as mild in 44 cases (93.6%) and moderate in three cases (6.4%), although no severe steatosis was found. Accordingly, the severity of steatosis was not considered in additional analyses.

Structural and functional vascular parameters in twins with NAFLD

Based on the ultrasonography examination, study subjects were divided into a group with any degree of sonographically detected steatosis (NAFLD, n = 47) and a control group with sonographically normal-appearing livers (non-NAFLD, n = 161) for additional analysis (Table 2). Twins in the NAFLD group were older than those in the normal group ($P < 0.001$). As expected, BMI was significantly higher in the NAFLD group ($P < 0.001$), while laboratory parameters of lipid metabolism and CRP levels showed no difference. Measurement of structural and vascular parameters commonly associated with increased risk of cardiovascular morbidity indicated that both brachial and aortic augmentation indices were significantly higher in twin subjects with NAFLD ($P < 0.001$). There was no significant distinction in the aortic pulse wave velocity between the groups ($P = 0.162$). IMT measurement was significantly increased in twin subjects with NAFLD at all standard locations ($P < 0.05$ - $P < 0.001$) except for the right distal common carotid artery ($P = 0.317$).

Heritability analysis of NAFLD in twins

The possible role of zygosity in the prevalence of NAFLD and its potential impact on the detection of abnormal vascular parameters in our twin cohort was estimated by age-adjusted base ACE analysis on 91 twin pairs after excluding opposite-sex dizygotic twin pairs Genetic and environmental variance estimates of this analysis are presented in Table 3. Accordingly, genetic factors do not appear to contribute to the presence or absence of NAFLD and the largest proportion of total variance are attributable to shared environmental factors. Upon further analysis, including adjustment to BMI, we found that unshared environmental effects have a moderate impact (25.8%) in this model.

Discussion

To our knowledge, this is the first twin study investigating the relative contribution of genetic and environmental factors to NAFLD. Genetic studies using the twin design are based upon the assumption that twins are representative of the general population for the outcomes being studied. Our analysis is based on data obtained from a fairly large cohort of healthy adult twin pairs and does not support an appreciable role for heritability of this liver disorder. We found that the overall prevalence of NAFLD, as determined by sonographic criteria, is similar to the general population with no difference according to zygosity. We also found that NAFLD is associated with older age, higher BMI and a series of abnormal vascular parameters such as higher peripheral and central augmentation indices and

Table 2. Association of structural and functional parameters with NAFLD in twins

	Total (n = 208)*	Non-NAFLD (n = 161)	NAFLD (n = 47)	P-value
Monozygotic : dizygotic	127 : 81	95 : 66	32 : 15	NS
Male : female	59 : 149	44 : 117	15 : 32	NS
Age, years	43.7 ± 16.7	41.5 ± 16.8	50.9 ± 14.3	<0.001
Weight, kg	72.6 ± 15.1	70.1 ± 14.1	80.5 ± 16.0	<0.001
Height, cm	167.3 ± 10.1	168.6 ± 9.9	163.3 ± 9.5	<0.001
BMI, kg/m^2	25.8 ± 5.0	24.6 ± 4.1	30.1 ± 5.2	<0.001
Total cholesterol, mmol/L	5.1 ± 0.9	5.1 ± 0.9	5.0 ± 0.9	NS
HDL cholesterol, mmol/L	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	NS
LDL cholesterol, mmol/L	3.2 ± 0.9	3.2 ± 0.8	3.1 ± 1.0	NS
Triglycerides, mmol/L	1.5 ± 0.9	1.4 ± 0.9	1.8 ± 0.8	NS
CRP, mg/L	2.2 ± 2.8	2.1 ± 2.9	2.6 ± 2.6	NS
Brachial AIx,%	−29.1 ± 33.1	−33.3 ± 33.0	−14.7 ± 29.6	<0.001
Aortic AIx,%	22.8 ± 16.7	20.7 ± 16.6	30.0 ± 15.0	<0.001
Aortic PWV, m/s	8.8 ± 3.9	8.6 ± 4.2	9.5 ± 2.7	NS
Right proximal CCA IMT, mm	0.59 ± 0.18	0.56 ± 0.16	0.70 ± 0.21	<0.001
Right distal CCA IMT, mm	0.69 ± 0.33	0.68 ± 0.34	0.73 ± 0.27	NS
Right ICA IMT, mm	0.60 ± 0.25	0.58 ± 0.22	0.67 ± 0.34	<0.05
Left proximal CCA IMT, mm	0.59 ± 0.17	0.57 ± 0.16	0.66 ± 0.20	<0.001
Left distal CCA IMT, mm	0.67 ± 0.25	0.65 ± 0.23	0.73 ± 0.27	<0.05
Left ICA IMT, mm	0.58 ± 0.22	0.56 ± 0.19	0.64 ± 0.29	<0.05

*104 twin pairs.

BMI, body mass index; AIx, augmentation index; PWV, pulse wave velocity; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C reactive protein; CCA, common carotid artery; ICA, internal carotid artery; IMT, intima media thickness; NS, not significant; NAFLD, Non-alcoholic fatty liver disease. Data are shown as mean ± standard deviation where appropriate.

Table 3. Age-adjusted univariate analysis of Non-alcoholic fatty liver disease heritability in twins

Measure	A	C	E
Non-BMI-corrected fatty liver	0.000 (0.000–0.537)	0.669 (0.147–0.923)	0.331 (0.117–0.653)
BMI-corrected fatty liver	0.000 (0.000–0.360)	0.742 (0.351–0.985)	0.258 (0.000–0.644)

Data shown are parameter estimates (95% confidence intervals) of univariate models. A, heritability; C, shared environmental variance component; E, unique environmental variance component; BMI, body mass index.

increased carotid IMT. However, we found no significant difference between monozygotic and dizygotic twins for the presence of sonographically detected NAFLD and any of the analysed laboratory parameters commonly linked to increased cardiovascular risk. These observations, therefore, fail to establish the role of genetic components in acquiring NAFLD and the cardiometabolic risk factors associated with this liver condition and investigated in this study.

There is increasing evidence suggesting that NAFLD may confer substantial cardiovascular risk independent of other components of the metabolic syndrome (4, 5). A growing number of reports have associated NAFLD with abnormal vascular parameters predicting the risk of cardiovascular disease (5, 6, 9). NAFLD may determine arterial stiffness based on studies describing increased carotid-femoral PWV and carotid IMT in subjects with this condition (25, 26). Our comprehensive approach of analysing carotid IMT bilaterally at three locations (proximal and distal CCA, ICA) indicates the same significant relationship. In addition, we observed that aortic PWV, as measured by oscillometry, tended to be higher in NAFLD subjects compared to non-NAFLD controls. In line with our findings, NAFLD has been associated with endothelial dysfunction based on findings of reduced flow-mediated dilatation (7). However, little has been known about altered augmentation indices in NAFLD patients until this report.

There are currently no literature data on the sensitivity of brachial and aortic augmentation index and aortic PWV assessed by oscillometry (TensioMed Arteriograph), although there are several publications concerning the validation of the device (18, 19). A recent study from Poland reported a sensitivity of 73.5% for carotid IMT values in patients with coronary artery disease and aortic stenosis (27). In a previous work we investigated the heritability of vascular parameters on a larger twin cohort and reported that low to moderate genetic variance is responsible for the determination of these traits, heritability ranging between 0 and 38% for carotid IMT values assessed at various anatomical sites, 45% for brachial augmentation index, and 42% for aortic PWV (28). Since we specifically wanted to see if these vascular parameters follow the NAFLD status, we can state that

these parameters seem phenotypically, but not genetically, associated.

We report no significant differences concerning the serum lipid parameters and CRP levels between non-NAFLD and NAFLD subjects. This is somewhat at variance with earlier observations, since triglycerides, in particular, have been found useful in predicting NAFLD severity (29). In our sample, we found higher levels of serum triglycerides in NAFLD subjects compared to non-NAFLD subjects (1.8 ± 0.8 vs. 1.4 ± 0.9), but the difference was not significant. However, it is important to recall that the degree of steatosis was mild in almost all analysed cases, which may serve as a possible explanation. This argument may also apply to other lipid parameters. Similar to our study, no correlation was observed between total or LDL cholesterol levels and the presence of fatty liver (17, 30), although lower HDL cholesterol levels have been reported in NAFLD (30). Finally, we found that NAFLD subjects had only a trend towards higher serum levels of CRP, which is an acute phase reactant often found to have increased levels in metabolic syndrome and NAFLD (31).

Heritability and the influence of shared and unshared environmental factors in the development of NAFLD and associated cardiometabolic risk factors are the key underlying questions of this article. Various twin studies have provided evidence that genetic factors play a considerable role in various cardiometabolic risk factors such as body weight regulation (32, 33). Few family or other non-twin studies have investigated the role of genetic factors in NAFLD, estimating a low to moderate heritability usually between 18 and 36% (11–13, 17). However, most participants in these studies were not healthy subjects but patients with various comorbidities. It must be taken into consideration that the family design is useful in the determination of inter-generation resemblance or difference, but, in contrast to the twin study design, it does not tangibly express outside factors, such as family environment and culture (34). Accordingly, family studies cannot reliably distinguish the heritability and common environmental effects. In contrast to the family study of Schwimmer and co-workers (13), inter-generation resemblance does not reside in genetic heritability based on our relatively large sample. By contrast, our findings indicate that common environmental factors are mostly responsible for the total variance of NAFLD. These shared environmental components estimate the contribution of a common family environment of twins including factors such as diet (as the most probable factor affecting the development of NAFLD), familiar socialization, exposure to high levels of air pollution and shared womb; whereas unshared environmental components account for factors affecting individual twins separately, such as smoking and physical activity, include measurement error and have a moderate impact.

Our study has several limitations. Steatosis can be visualized using ultrasonography when the liver shows homogeneously increased echogenicity compared to the adjacent right renal parenchyma. This relatively inexpensive imaging method has been successfully used to screen large populations for the presence of fatty liver (35). The sensitivity of ultrasonography to detect steatosis is 60–94% (36) and may reach 100% if the amount of liver fat exceeds 33% (16). If steatosis is mild, however, the sensitivity may drop to 53% (37), indicating that mild cases of NAFLD may have been missed in our study. Moreover, ultrasonography provides a semi-quantitative estimate of liver fat content and cannot distinguish steatosis from steatohepatitis, a task for which liver biopsy may be required (36, 37). This limitation introduces additional variability to the definition of NAFLD in our study. Another weakness of ultrasonography is its operator dependency (36). However, all subjects in our study were evaluated by a single sonographer, omitting therefore the problem of inter-observer variability.

Additional limitations include the relatively small number of participating dizygotic twins compared to the usual twin study design, which may lead to statistical errors in the ACE analysis. Furthermore, few subjects had moderate steatosis and no severe steatosis was found, with NAFLD being defined in most subjects by mild steatosis only. Consequently, the power of the study to yield differences by comparing NAFLD and non-NAFLD groups has been further limited and may account for nonsignificant difference of some of the analysed variables.

A major strength of the present study is that the tests were performed on twins to complement previous family studies conducted in this field. In addition, carotid IMT values were measured on three locations bilaterally and augmentation indices were obtained, resulting in comprehensive vascular evaluation of an essentially healthy cohort. We may reasonably assume that the twins enrolled did not differ from the non-twin individuals with regard to the traits considered, and the results can therefore be generalized to the singleton population.

In conclusion, our study suggests a negligible role within a healthy twin population for the heritability of NAFLD as a condition defined within the limitations of sonographic criteria. In addition, shared and unshared environmental effects, respectively, account for 74.2 and 25.8% of the studied variations in coexisting changes in structural and functional vascular parameters commonly associated with increased cardiovascular risk. The findings support the view that NAFLD and the vascular changes associated with this disorder may be first and foremost acquired through environmental effects, underscoring the importance of lifestyle in primary prevention.

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