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Childhood predictors of adult fatty liver. The Cardiovascular Risk in Young Finns Study

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Background & Aims: Fatty liver is a potentially preventable cause of serious liver diseases. This longitudinal study aimed to identify childhood risk factors of fatty liver in adulthood in a population-based group of Finnish adults.

Methods: Study cohort included 2,042 individuals from the Cardiovascular Risk in Young Finns Study aged 3–18 years at baseline in 1980. During the latest follow-up in 2011, the liver was scanned by ultrasound. In addition to physical and environmental factors related to fatty liver, we examined whether the genetic risk posed by a single nucleotide polymorphism in the patatin-like phospholipase domain-containing protein 3 gene (*PNPLA3*) (rs738409) strengthens prediction of adult fatty liver.

Results: Independent childhood predictors of adult fatty liver were small for gestational age, (odds ratio = 1.71, 95% confidence interval = 1.07–2.72), variant in *PNPLA3* (1.63, 1.29–2.07 per one risk allele), variant in the transmembrane 6 superfamily 2 gene (*TM6SF2*) (1.57, 1.08–2.30), BMI (1.30, 1.07–1.59 per standard deviation) and insulin (1.25, 1.05–1.49 per standard deviation). Childhood blood pressure, physical activity, C-reactive protein, smoking, serum lipid levels or parental lifestyle factors did not predict fatty liver. Risk assessment based on childhood age, sex, BMI, insulin levels, birth weight, *TM6SF2* and *PNPLA3* was superior in predicting fatty liver compared with the approach using only age, sex, BMI and insulin levels (C statistics, 0.725 *vs.* 0.749; *p* = 0.002).

Conclusions: Childhood risk factors on the development of fatty liver were small for gestational age, high insulin and high BMI. Prediction of adult fatty liver was enhanced by taking into account genetic variants in *PNPLA3* and *TM6SF2* genes.

Lay summary: The increase in pediatric obesity emphasizes the importance of identification of children and adolescents at high risk of fatty liver in adulthood. We used data from the longitudinal Cardiovascular Risk in Young Finns Study to examine the associations of childhood (3–18 years) risk variables with fatty liver assessed in adulthood at the age of 34–49 years. The findings suggest that a multifactorial approach with both lifestyle and genetic factors included would improve early identification of children with a high risk of adult fatty liver.

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Introduction

Fatty liver without excessive alcohol intake is the most common form of chronic liver disease in Western countries with prevalence between 20–30% and 70–90% in the obese and diabetics [1]. One of the major modifiable risk factors for fatty liver disease is obesity which often begins in childhood [2,3]. There is no

effective cure for advanced fatty liver, and thus the increase in pediatric obesity emphasizes the importance of identification of children and adolescents at high risk of fatty liver in adulthood.

Fatty liver is the result of accumulation of triacylglycerol in the hepatocytes [4–7]. Although evidence that hepatic fat accumulation determines insulin resistance is still lacking in humans [7], it has been hypothesized that hepatic accumulation of the diacylglycerols may lead to activation of protein kinase Cε, resulting in hepatic insulin resistance. Additionally, intracellular compartmentation of diacylglycerols is a critical factor in determining whether increased hepatic diacylglycerol content results in hepatic insulin resistance and will likely explain why some patients with fatty liver are not associated with hepatic insulin resistance [8,9]. Besides obesity and diabetes, recently also genetic factors have been shown to be associated with fatty liver [7]. Romeo *et al.* [10] were the first to report that the rs738409 C>G SNP in the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene, encoding the isoleucine to methionine variant at protein position 148, was strongly associated with increased liver fat content [10]. Since then, several other pieces of evidence have highlighted the major role of *PNPLA3* in the development and progression of NAFLD [11,12]. Mutated *PNPLA3* variant is attached on the surface of lipid droplets reducing triglyceride breakdown leading to lipid retention in the hepatocyte lipid droplet [7]. In addition to *PNPLA3*, we examined other single nucleotide polymorphisms that have been linked to fatty liver: *TM6SF2, GCKR* and *LYPLAL1* [12]. The low-frequency rs58542926 C>T polymorphism of *TM6SF2* encodes the loss-of-function E167K variant, which leads to reduced secretion of very low-density lipoproteins (VLDL) resulting in intrahepatic retention of triglycerides and steatosis [13–17]. The common glucokinase regulator (*GCKR*) regulates glucose uptake by hepatocytes [18]. In this study, we used the rs1260326 encoding for the *P446L* protein variant. The *P446L* variant affects *GCKR* ability to negatively regulate glucokinase in response to fructose-6-phosphate, thereby determining constitutive activation of hepatic glucose uptake [19]. Single nucleotide polymorphism in the lysophospholipase-like 1 locus (*LYPLAL1*, rs12137855) encodes an enzyme likely involved in triglycerides catabolism in the liver.

The present study aimed to identify the childhood physical and environmental predictors of adult fatty liver. We used data from the longitudinal Cardiovascular Risk in Young Finns Study to examine the associations of childhood (3–18 years) risk variables with fatty liver assessed in adulthood at the age of 34–49 years. We also examined whether adding information on the genetic variants in *PNPLA3, TM6SF2, GCKR* and *LYPLAL1* enhances early identification of children who may be at risk for adult fatty liver.

Materials and methods

Study population

The Cardiovascular Risk in Young Finns Study is an ongoing population-based follow-up study of atherosclerotic precursors. In 1980, a total of 4,320 Finnish children in 6 age cohorts (3, 6, 9, 12, 15, and 18 years of age) were invited, and 3,596 (83.2%) participated in the first cross-sectional survey [20]. Participants were randomly chosen from the national population register. Since then, follow-ups have been conducted in the whole population in 1983, 1986, 2001, 2007 and 2011. In the latest follow-up in 2011, a total of 2,042 (age, 34–49 years) participants were reexamined. All participants provided written informed con-

sent, and the study was approved by Ethics Committee of Hospital District of Southwest Finland in agreement with the Declaration of Helsinki.

Ultrasound imaging of liver

Ultrasound imaging of the liver was performed for 2,042 study participants using a validated protocol [21] and Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA) with 4.0 MHz adult abdominal transducers. Evaluation of hepatic steatosis was performed according to liver-to-kidney contrast, parenchymal brightness, deep beam attenuation and bright vessel walls [22]. According to these criteria the presence of hepatic steatosis was assessed visually by a one trained ultrasonographer masked to participant's characteristics.

Clinical characteristics

Height and weight were measured and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured from the brachial artery with a standard mercury sphygmomanometer. The average of three measurements was used in statistical analysis.

Questionnaires were used to obtain data on smoking, age at menarche, physical activity, birth weight, birth height, length of gestation, breastfeeding, family history of coronary heart disease, parental hypertension (self-reported diagnosis of hypertension in either parent at baseline) and parental occupational status. Data on birth weight and birth height was verified by well-baby clinic records. In 1980, 1983 and 1986, questionnaire information on cigarette smoking was collected in participants aged 12 years or older. Individuals who had reported daily smoking at any age between ages 12 and 18 were defined as smokers. Physical activity was available in participants aged 9 years or older. The physical activity index was calculated as previously described (range 5–15) [23]. In 2001, 2007 and 2011 follow-ups, adulthood alcohol consumption data were acquired by standardized questionnaires. Excess alcohol intake was defined as consuming ⩾6 alcohol doses all at once at least once a week. By using data collected from national hospital discharge registries were able to verify that none of the participants had viral or autoimmune causes of fatty liver. Furthermore, all results remained similar after excluding participants with history of cancer (N = 4) and psychotic disorders (N = 3), who may potentially have medications influencing liver fat metabolism.

Venous blood samples were drawn after an overnight fast for determination of serum lipid levels, insulin, and CRP. Serum insulin was measured with immunoassay [24]. Standard enzymatic methods were used for serum total cholesterol, triglycerides, and high-density lipoprotein cholesterol [25,26]. Low-density lipoprotein cholesterol concentration was calculated by the Friedewald formula in subjects with triglycerides <4.0 mmol/L. Serum high-sensitivity C-reactive protein (CRP) was analyzed by immunoassay [27].

Genetic analyses

In the present study, we used the SNPs rs738409 near the *PNPLA3* gene, rs58542926 near the *TM6SF2* gene, rs12137855 near the gene *LYPLAL1* and rs780094 near the *GCKR* gene, associated with fatty liver in recently genome-wide association analyses [10,12,13,15–17,28], as the genetic marker for susceptibility for fatty liver. Genotyping was performed with the custom-built Illumina BeadChip 670K. Missing genotypes have been imputed to the 1,000 genomes reference panel and imputed single nucleotide polymorphism have been filtered based on low call-rate (<0.95), low-information score (<0.4), minor allele frequency <1%, and deviation from Hardy-Weinberg equilibrium ($p < 5.0 \times 10^{-6}$).

Statistical analyses

The participants were classified into fatty liver and normal liver groups. The risk allele count for the SNP rs738409 was coded 0/1/2, where 2 denotes a GG and 1 a GC genotype. Birth weight and height were treated as continuous variables. Preterm birth was defined as birth before 37 weeks' gestation. Small for gestational age was defined as birth weight below the 10th percentile, appropriate birth weight for gestational age as birth weight in the 50–90th percentiles and large for gestational age as birth weight over the 90th percentile. At baseline, the participants were classified as smokers if they smoked daily. Parental occupational status was divided into 3 categories: manual, lower-grade non-manual, and higher-grade non-manual. Values for triglycerides and CRP were log-transformed before analyses because of skewed distributions. The distribution of physical activity index was strongly skewed. Thus, the physical activity index was divided to quartiles. Alcohol consumption data were calculated in standard

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doses (12 g pure ethanol) per day by dividing the total number of doses consumed per week (0.33 L doses of beer or cider, 0.12 L doses of wine, and 0.04 L doses of hard liquor) by 7.

An attrition analysis was performed to determine whether the representativeness of the baseline sample was maintained in the present cohort; baseline characteristics were compared between those who participated and those who did not participate at follow-up.

Logistic regression was used to examine the odds ratios (OR) of adult fatty liver for a 1-SD increase for each of the continuous childhood variables. The variables that were significant in age-, sex- and BMI-adjusted analyses were then added to a multivariable logistic regression model to determine the independent childhood predictors of adult fatty liver. To examine whether gene variant status modifies the associations between the predictor variables and fatty liver, we included predictor variable x gene variant interaction terms in regression models. The incremental value of adding risk variables to predict adult fatty liver was examined through the use of alcohol consumption adjusted multivariate logistic regression models. Additionally, birth weight was selected to the final multivariable model and model comparison analysis, and the ORs of birth height, small for gestational age and preterm birth were assessed in separate models. The ability of several risk variables to predict fatty liver risk was estimated with alcohol consumption adjusted C statistics by calculating the area under the receiver-operating characteristic curve, the net reclassification improvement, and the integrated discrimination index [29]. The net reclassification improvement and integrated discrimination index were calculated to determine the extent to which incorporation of birth weight and the genetic variant in *PNPLA3* reassigned individuals to risk categories that more correctly reflected whether the subjects developed fatty liver in adulthood.

Two-tailed *p* values less than 0.05 were considered statistically significant. All statistical analyses were performed with the Statistical Analysis System 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics of the study participants (n = 2,042) are shown in Table 1. The prevalence of adult fatty liver was 19% (n = 385). To determine whether the representativeness of the baseline sample was maintained in the present cohort, we compared the baseline characteristics between those who participated and those who did not participate at follow-up. Non-participants were younger (9.9 *vs.* 10.9 years; *p* <0.0001, sex-adjusted analysis of variance) and more often male (54% *vs.* 45%; *p* <0.0001, Chi-squared test) than participants. No statistically significant differences were observed for other baseline study variables.

Childhood risk factors for adult fatty liver

Age-, sex- and BMI-adjusted ORs and confidence intervals (CI) for adult fatty liver according to childhood variables are shown in Table 2. Male sex, preterm birth, small for gestational age, *TM6SF2*, *PNPLA3*, age- and sex-adjusted childhood BMI and insulin levels, low birth weight and low birth height showed significant ORs for adult fatty liver.

Multivariable logistic regression models adjusted with adulthood long term alcohol consumption were constructed to examine the independent contributions of childhood risk variables to the development of the adult fatty liver. Significant variables from the Table 2 were selected in the models. In a model containing age, sex, insulin, BMI, birth weight, *TM6SF2* and *PNPLA3*, the independent predictors of adult fatty liver included male sex, variant in *PNPLA3*, variant in *TM6SF2,* insulin, BMI and birth weight (Table 3). We also determined in separate multivariable models the ORs for small for gestational age (OR 1.71, 95% CI 1.07–2.72, *p* = 0.02), birth height (0.87, 0.75–1.01, *p* = 0.06) and preterm birth (2.03, 1.08–3.84, *p* = 0.03). In the interaction anal-

Table 1. **Baseline characteristics of participants with and without fatty liver diagnosis in adulthood (N = 2,042).**

Adult fatty liver status in 2011	Normal liver	Fatty liver	*p* value
No.	1657	385	
Age (yr)	10.7 ± 0.1	12.3 ± 0.3	<0.0001
Male sex (%)	40.0	68.3	<0.0001
BMI (kg/m^2)	17.7 ± 0.1	19.0 ± 0.2	<0.0001
z-BMI	-0.07	0.14	<0.0001
Systolic blood pressure (mmHg)	112.1 ± 0.3	115.7 ± 0.6	<0.0001
Diastolic blood pressure (mmHg)	68.3 ± 0.3	69.7 ± 0.5	0.01
Total cholesterol (mmol/L)	5.3 ± 0.0	5.1 ± 0.0	0.0006
LDL cholesterol (mmol/L)	3.5 ± 0.0	3.3 ± 0.0	0.0008
HDL cholesterol (mmol/L)	1.6 ± 0.0	1.5 ± 0.0	0.03
Triglycerides (mmol/L)	0.66 ± 0.0	0.70 ± 0.0	0.03
Insulin (mU/L)	9.5 ± 0.1	11.4 ± 0.3	<0.0001
CRP (mg/L)	1.0 ± 0.1	1.0 ± 0.2	0.9
Birth weight (g)	3529 ± 15	3441 ± 32	0.01
Birth height (cm)	50.4 ± 0.1	50.2 ± 0.1	0.2
Large for gestational age (%)	9.4	8.8	0.7
Small for gestational age (%)	9.1	14.8	0.005
Appropriate for gestational age (%)	81.5	76.4	0.06
Preterm birth (%)	3.1	6.9	0.002
Breast-feeding (%)[1]	85.9	78.8	0.005
Daily smoking (%)[a]	18.5	23.6	0.03
Physical activity index	8.4 ± 0.1	8.6 ± 0.1	0.3
Mother's BMI	23.8 ± 0.1	24.7 ± 0.2	<0.0001
Father's BMI	25.4 ± 0.1	25.8 ± 0.2	0.05
Parental occupational status (%)*	38/43/19	44/42/14	0.03
Parental hypertension prevalence (%)	13.6	20.1	0.003
Age at menarche (yr)[a]	13.1 ± 0.1	12.7 ± 0.1	0.02
Variant in *PNPLA3* (CC/CG/GG) (%)	60/36/4	54/38/8	0.003
Variant in *TM6SF2* (CC/CT/TT) (%)	90/10/0	82/17/1	0.002
Variant in *GCKR* (CC/CT/TT) (%)	42/46/12	38/43/19	0.004
Variant in *LYPLAL1* (CC/CT/TT) (%)	53/41/6	50/41/9	0.1

BMI, body mass index; CRP, C reactive protein; CHD, coronary heart disease. Values are least squares means ± standard error for continuous variables and percentages for categorical variables. [1]Breastfed = 1, not breastfed = 0. *Three categories according to a parental occupation (manual, lower-grade nonmanual, higher-grade nonmanual). [a]Data available for 12–18 years old participants. z-BMI, age and sex adjusted body mass index z-score.

yses, there were no statistically significant predictor variable x gene variant interactions. Excluding participants with excess alcohol intake did not change the results.

Multiple childhood risk factors in predicting adult fatty liver

We studied the ability of multiple childhood risk factors to predict adult fatty liver with several models (Table 4). Models were adjusted with long term alcohol consumption. A model that included age, sex, BMI, insulin and birth weight (model 2) performed better than a model including only age, sex, BMI, and insulin (model 1) (*p* = 0.03 model 2 *vs.* 1). When both *PNPLA3* and *TM6SF2* were included in the model (model 3), the area under the curve value increased significantly (*p* = 0.002 when compared to model 1, and *p* = 0.05 when compared to model 2). Consistent improvements were also seen for the net reclassification improvement and integrated discrimination index between mod-

Table 2. Age-, sex- and BMI-adjusted odds ratios and 95% confidence intervals for adult fatty liver for each of the youth variables (N = 2,027).

Variable	OR	95% CI	p value
Male sex (age- and BMI-adjusted)[#]	3.33	2.62-4.23	<0.0001
Preterm birth[#]	2.41	1.37-4.25	0.002
Small for gestational age[#]	1.75	1.16-2.63	0.008
Variant in TM6SF2*	1.71	1.23-1.37	0.002
Variant in PNPLA3*	1.38	1.12-1.69	0.003
BMI (age- and sex-adjusted)[§]	1.34	1.15-1.55	0.0001
Insulin[§]	1.29	1.12-1.47	0.0003
Parental hypertension[#]	1.25	0.89-1.75	0.2
Breast-feeding[#]	1.29	0.80-2.07	0.3
Variant in LYPLAL1*	1.15	0.95-1.41	0.2
Diastolic blood pressure[§]	1.06	0.94-1.21	0.4
Daily smoking [a][#]	1.04	0.78-1.40	0.8
Age (sex- and BMI-adjusted)[§]	1.03	1.00-1.07	0.06
Mother´s BMI[§]	1.03	1.00-1.06	0.08
Systolic blood pressure[§]	1.03	0.89-1.19	0.7
Father´s BMI[§]	1.01	0.97-1.06	0.5
CRP[§]	1.00	0.96-1.04	1
HDL cholesterol[§]	0.98	0.87-1.10	0.7
Triglycerides[§]	0.98	0.87-1.10	0.7
Physical activity index[‖]	0.97	0.79-1.19	0.8
Total cholesterol[§]	0.90	0.80-1.02	0.09
LDL cholesterol[§]	0.89	0.79-1.01	0.06
Large for gestational age[#‖]	0.87	0.53-1.41	0.6
Parental occupational status [c]	0.85	0.71-1.02	0.07
Variant in GCKR*	0.83	0.70-1.00	0.05
Birth height[§]	0.82	0.72-0.94	0.003
Age at menarche [a, b]	0.80	0.63-1.01	0.06
Birth weight[§]	0.77	0.68-0.88	<0.0001
Appropriate for gestational age[#]	0.76	0.55-1.06	0.1

Values of BMI, systolic and diastolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, insulin, CRP, birth weight, birth height and physical activity index were standardized. OR, odds ratio; CI, confidence interval. [#]OR for categorical predictor. *OR per risk allele. [§]OR for a 1-SD increase. [‖]OR for a one quartile increase in physical activity index or for parental occupation category. [a]Data only available for 12–18 years old participants. [b]Only for girls. [c]Three categories according to a parental occupation (manual, lower-grade non-manual, higher-grade non-manual).

els 1 and 3. Notably, the net reclassification improvement for model 3 was >28% with respect to the model including only age, sex, BMI and insulin. Excluding participants with excess alcohol intake did not change the results.

Discussion

In the present study, we observed that the prediction of fatty liver in adulthood can be improved significantly by the use of single nucleotide polymorphism in the PNPLA3 and TM6SF2 genes compared with prediction models consisting of only age, sex, and childhood BMI and insulin levels. Our results also suggest that the prediction of adult fatty liver is improved by the use of data on birth weight.

In this study, we were able to take into account several potential childhood risk factors for adult fatty liver in a large cohort of children followed up over 30 years. We have recently shown in this cohort that adulthood determinants of fatty liver are waist circumference, alanine aminotransferase, BMI, male sex,

Table 3. Multivariable odds ratios of childhood risk factors for adult fatty liver (N = 1,456).

Variable	OR	95% CI	p value
Male sex	3.33	2.41-4.61	<0.0001
Variant in PNPLA3 (per G allele)	1.63	1.29-2.07	<0.0001
Variant in TM6SF2 (per T allele)	1.57	1.08-2.30	0.02
BMI	1.30	1.07-1.59	0.009
Insulin	1.25	1.05-1.49	0.01
Age	1.02	0.98-1.06	0.4
Birth weight	0.81	0.70-0.93	0.004

Values of birth weight, birth height, BMI and insulin were standardized. Age-, sex- and BMI-adjusted significant variables were selected in the model. OR, odds ratio; CI, confidence interval. OR for a 1-SD increase. Adjusted with adulthood long term alcohol consumption.

apolipoprotein B, systolic blood pressure, alcohol intake, insulin and low physical activity index [30]. Insulin and BMI were significant predictors of fatty liver already in childhood. However, early life exposure to elevated systolic blood pressure and low physical activity did not predict adult fatty liver. This emphasizes the role of childhood BMI as a modifiable risk factor for adult fatty liver.

High childhood insulin level was an independent predictor of adult fatty liver. A potential explanation is that liver fat accumulation occurs when hyperinsulinemia and insulin resistance lead to hepatic accumulation of triglycerides. This process usually results from an imbalance between increased free fatty acid flux from adipose tissue to the liver, increased caloric intake, and increased lipogenesis in the liver and the liver's handling and export of the extra fat. The free fatty acids are usually either oxidized in the mitochondria or esterified to triglycerides, which in turn are either packaged as VLDL for export or are used for the production of lipids [31].

Interestingly, low birth weight was an independent predictor of adult fatty liver. This is in line with one previous study of 2,003 Finnish adults: a significant association between adulthood liver fat score (based on five variables: presence of metabolic syndrome, presence of type 2 diabetes, fasting serum insulin and aspartate aminotransferase levels, and aspartate aminotransferase/alanine aminotransferase ratio) and birth weight was seen in women [32]. This may be influence of rapid weight catch-up growth [33,34]. Catch-up growth is recognized as a major risk factor for later development of insulin-related complications and chronic diseases like abdominal obesity, type 2 diabetes and cardiovascular disease [35]. In a longitudinal study of 51 Spanish children, small for gestational age children gained progressively more body fat and abdominal fat mass than appropriate for gestational age children between ages 2 and 4 years. These differences occurred despite the small for gestational age children having already completed their catch-up growth and weight gain by age 2 years [36]. On the other hand, low birth weight was associated with adult fatty liver even when adjusted with childhood BMI. This could be explained by the model for the origins of disease that proposes that nutrition during fetal life, infancy, and early childhood establish gene expression and thereby permanently set functional capacity, metabolic competence, and responses to the later environment [37]. Furthermore, it is known that liver growth may be reduced as part of the adaptive response to a poor fetal substrate supply [38].

In this study, we used the genetic variants in PNPLA3, TM6SF2, GCKR and LYPLAL1 genes recognized to being involved in determining the risk of fatty liver [7,39]. The value of these novel

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Table 4. Comparison of models for the prediction of adult fatty liver in 1,456 individuals.

	AUC value	p value	NRI, %	p value	IDI, %	p value	H-L	p value	Model used for comparison
Model 1									
Age, sex, BMI, insulin	0.725						2.1	1	
Model 2									
Model 1 + birth weight	0.736	0.03	12.7	0.06	6.5	0.03	6.8	0.6	1
Model 3									
Model 1 + birth weight, *PNPLA3* and *TM6SF2*	0.749	0.002	28.6	<0.0001	23.5	<0.0001	11.9	0.2	1
		0.05	24.6	0.0003	16.0	<0.0001			2

AUC, area under the curve; CI, confidence interval; NRI, net reclassification improvement; IDI, integrative discrimination index; H-L, Hosmer-Lemeshow x2 statistics. Adjusted with adulthood long term alcohol consumption.

genetic markers in identifying young individuals at risk for adult fatty liver disease is unknown. We observed that a combined risk score based on childhood BMI and insulin levels, birth weight and the genetic variants in *PNPLA3* and *TM6SF2* was superior to a model including only BMI and insulin values in predicting adult fatty liver. In *PNPLA3* gene, rs738409 is a C/G single nucleotide variation on human chromosome 22. A cytosine to guanine DNA substitution (rs738409) encodes an isoleucine to methionine loss-of-function substitution at the amino acidic residue 148 of the *PNPLA3* protein [40]. Mutated *PNPLA3* variant is attached on the surface of lipid droplets reducing triglyceride breakdown leading to lipid retention in the hepatocyte lipid droplet [7,41]. Alternatively, it may stimulate the hepatic triglycerides synthesis [42,43]. The variant increases the risk of fatty liver, but is not associated with increased risk of type 2 diabetes or cardiovascular disease [44]. However, as the carriers of the variant allele are at high risk of developing advanced liver disease, they may be an important target group for lifestyle intervention [45]. The low-frequency rs58542926 polymorphism of TM6SF2 encodes the loss-of-function E167K variant that has been linked with fatty liver and lower serum lipoproteins [7]. The mechanism is related to reduced secretion of VLDL resulting in intrahepatic retention of triglycerides [7]. Altogether, the present data suggest that a multifactorial approach if implemented could improve the identification of children with a high risk of adult fatty liver. Moreover, these data demonstrate that the prediction of adult fatty liver was enhanced by taking into account genetic variants.

Strengths and limitations

We studied a large, randomly selected, and carefully phenotyped cohort of young men and women prospectively followed up for up to 31 years since early childhood. Extensive data were available on several possible childhood physical, environmental, and genetic determinants of fatty liver that could be comprehensively taken into account in multivariable models. Because our study cohort was homogeneous ethnic group, the generalizability of our results is limited to Caucasians. The limitations of this study include the loss of original participants during the long term follow-up. We found that non-participants were younger and more often male in childhood than participants. Therefore, the rates of adult fatty liver in our cohort might be an underestimation of the real rates. One limitation of our study was that we did not have data on liver enzymes measured in childhood.

Liver biopsy is at present the gold standard for diagnosing fatty liver, because it is the only way to detect inflammation or fibrosis [46]. However, it is an invasive procedure with potential risk of bleeding, and it is not suitable for large-scale population studies of fatty liver [47,48]. Ultrasound imaging has also its limitations: while the test shows high specificity, it has low sensitivity [49]. It has been estimated that conventional ultrasound imaging can only detect steatosis when more than 30% of the liver is affected [50]. However, a large meta-analysis [51] concluded that ultrasonography allows for reliable and accurate detection of moderate-severe fatty liver, compared to histology. Liver ultrasound is an operator-dependent modality with varying results between operators. In the meta-analysis by Hernaez *et al.*, the range of kappa values for intra-rater evaluation was 0.54–0.92 [51]. In our study, all ultrasound images were graded by one trained operator who was masked for participant's clinical characteristics. Because ultrasound imaging is non-invasive, widely accessible, and cost-effective, it is likely a reasonable choice for population-based studies into the etiogenesis of fatty liver [51].

Conclusions

We found that low birth weight, the SNP rs738409 in the *PNPLA3* gene, the SNP rs58542926 in the TM6SF2 gene and insulin and BMI levels measured in childhood were independently related to fatty liver detected 31 years later in adulthood. Including information on birth weight, rs738409 and rs58542926 in addition to childhood BMI and insulin levels significantly improved the ability of the statistical model to predict adult fatty liver. Therefore, the present findings suggest that a multifactorial approach if implemented would improve early identification of children with a high risk of adult fatty liver.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors' contributions

Emmi Suomela: study concept and design; analysis and interpretation of data; statistical analysis; drafting of the article. Mervi Oikonen: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the article; critical revision of the manuscript for important intellectual content; study supervision. Niina Pitkänen: analysis and interpretation of data; drafting of the article; critical revision of the manuscript for important intellectual content. Ari Ahola-Olli: analysis and interpretation of data; drafting of the article; critical revision of the manuscript for important intellectual content. Johanna Virtanen: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Riitta Parkkola: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Eero Jokinen: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Tomi Laitinen: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Nina Hutri-Kähönen: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Mika Kähönen: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Terho Lehtimäki: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Leena Taittonen: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Päivi Tossavainen: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Antti Jula: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Britt-Marie Loo: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Vera Mikkilä: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Risto Telama: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Jorma S. A. Viikari: study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Markus Juonala: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. Olli T. Raitakari: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the article; critical revision of the manuscript for important intellectual content; study supervision.

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Supplementary data

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References

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[1] **Targher G**, **Day CP**, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med 2010;363:1341–1350.

[2] Alisi A, Manco M, Vania A, Nobili V. Pediatric nonalcoholic fatty liver disease in 2009. J Pediatr 2009;155:469–474.

[3] Patton HM, Sirlin C, Behling C, Middleton M, Schwimmer JB, Lavine JE. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. J Pediatr Gastroenterol Nutr 2006;43:413–427.

[4] Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221–1231.

[5] Reue K. A thematic review series: lipid droplet storage and metabolism: from yeast to man. J Lipid Res 2011;52:1865–1868.

[6] Quiroga AD, Lehner R. Liver triacylglycerol lipases. Biochim Biophys Acta 1821;2012:762–769.

[7] Dongiovanni P, Romeo S, Valenti L. Genetic factors in the pathogenesis of nonalcoholic fatty liver and steatohepatitis. Biomed Res Int 2015;2015:460190.

[8] Byrne CD. Ectopic fat, insulin resistance and non-alcoholic fatty liver disease. Proc Nutr Soc 2013;72:412–419.

[9] Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology 2014;59:713–723.

[10] **Romeo S**, **Kozlitina J**, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461–1465.

[11] Macaluso FS, Maida M, Petta S. Genetic background in nonalcoholic fatty liver disease: A comprehensive review. World J Gastroenterol 2015;21:11088–11111.

[12] Dongiovanni P, Valenti L. Genetics of nonalcoholic fatty liver disease. Metabolism 2015.

[13] **Kozlitina J**, **Smagris E**, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46:352–356.

[14] **Holmen OL**, **Zhang H**, **Fan Y**, Hovelson DH, Schmidt EM, Zhou W, et al. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. Nat Genet 2014;46:345–351.

[15] Liu YL, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:4309.

[16] **Dongiovanni P**, **Petta S**, Maglio C, Fracanzani AL, Pipitone R, Mozzi E, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. Hepatology 2015;61:506–514.

[17] Sookoian S, Castano GO, Scian R, Mallardi P, Fernandez Gianotti T, Burgueno AL, et al. Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. Hepatology 2015;61:515–525.

[18] Petta S, Miele L, Bugianesi E, Camma C, Rosso C, Boccia S, et al. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. PLoS One 2014;9 e87523.

[19] Beer NL, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orho-Melander M, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. Hum Mol Genet 2009;18:4081–4088.

[20] Raitakari OT, Juonala M, Rönnemaa T, Keltikangas-Järvinen L, Räsänen L, Pietikäinen M, et al. Cohort profile: the Cardiovascular Risk in Young Finns Study. Int J Epidemiol 2008;37:1220–1226.

[21] Edens MA, van Ooijen PM, Post WJ, Haagmans MJ, Kristanto W, Sijens PE, et al. Ultrasonography to quantify hepatic fat content: validation by 1H magnetic resonance spectroscopy. Obesity (Silver Spring) 2009;17:2239–2244.

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[22] Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. Br Med J (Clin Res Ed) 1986;292:13–15.

[23] Telama R, Viikari J, Välimäki I, Siren-Tiusanen H, Åkerblom HK, Uhari M, et al. Atherosclerosis precursors in Finnish children and adolescents. X. Leisure-time physical activity. Acta Paediatr Scand Suppl 1985;318:169–180.

[24] Herbert V, Lau KS, Gottlieb CW, Bleicher SJ. Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 1965;25:1375–1384.

[25] Porkka KV, Raitakari OT, Leino A, Laitinen S, Räsänen L, Rönnemaa T, et al. Trends in serum lipid levels during 1980–1992 in children and young adults. The Cardiovascular Risk in Young Finns Study. Am J Epidemiol 1997;146:64–77.

[26] Juonala M, Viikari JS, Hutri-Kähönen N, Pietikäinen M, Jokinen E, Taittonen L, et al. The 21-year follow-up of the Cardiovascular Risk in Young Finns Study: risk factor levels, secular trends and east-west difference. J Intern Med 2004;255:457–468.

[27] Juonala M, Viikari JS, Rönnemaa T, Taittonen L, Marniemi J, Raitakari OT. Childhood C-reactive protein in predicting CRP and carotid intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. Arterioscler Thromb Vasc Biol 2006;26:1883–1888.

[28] **Speliotes EK**, **Yerges-Armstrong LM**, **Wu J**, **Hernaez R**, **Kim LJ**, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 2011;7 e1001324.

[29] Pencina MJ, D'Agostino Sr RB, D'Agostino Jr RB, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157–172, [Discussion 207–212].

[30] Suomela E, Oikonen M, Virtanen J, Parkkola R, Jokinen E, Laitinen T, et al. Prevalence and determinants of fatty liver in normal-weight and overweight young adults. The Cardiovascular Risk in Young Finns Study. Ann Med 2014:1–7.

[31] Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. Science 2011;332:1519–1523.

[32] Sandboge S, Perälä MM, Salonen MK, Blomstedt PA, Osmond C, Kajantie E, et al. Early growth and non-alcoholic fatty liver disease in adulthood-the NAFLD liver fat score and equation applied on the Helsinki Birth Cohort Study. Ann Med 2013;45:430–437.

[33] Breij LM, Kerkhof GF, Hokken-Koelega AC. Accelerated infant weight gain and risk for nonalcoholic fatty liver disease in early adulthood. J Clin Endocrinol Metab 2014;99:1189–1195.

[34] Faienza MF, Brunetti G, Ventura A, D'Aniello M, Pepe T, Giordano P, et al. Nonalcoholic fatty liver disease in prepubertal children born small for gestational age: influence of rapid weight catch-up growth. Horm Res Paediatr 2013;79:103–109.

[35] Dulloo AG. Regulation of fat storage via suppressed thermogenesis: a thrifty phenotype that predisposes individuals with catch-up growth to insulin resistance and obesity. Horm Res 2006;65:90–97.

[36] Ibanez L, Ong K, Dunger DB, de Zegher F. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab 2006;91:2153–2158.

[37] Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311:171–174.

[38] Morrison JL, Duffield JA, Muhlhausler BS, Gentili S, McMillen IC. Fetal growth restriction, catch-up growth and the early origins of insulin resistance and visceral obesity. Pediatr Nephrol 2010;25:669–677.

[39] Marzuillo P, Del Giudice EM, Santoro N. Pediatric non-alcoholic fatty liver disease: New insights and future directions. World J Hepatol 2014;6:217–225.

[40] **Pingitore P**, **Pirazzi C**, Mancina RM, Motta BM, Indiveri C, Pujia A, et al. Recombinant PNPLA3 protein shows triglyceride hydrolase activity and its I148M mutation results in loss of function. Biochim Biophys Acta 2014;1841:574–580.

[41] Dongiovanni P, Donati B, Fares R, Lombardi R, Mancina RM, Romeo S, et al. PNPLA3 I148M polymorphism and progressive liver disease. World J Gastroenterol 2013;19:6969–6978.

[42] Donati B, Motta BM, Pingitore P, Meroni M, Pietrelli A, Alisi A, et al. The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. Hepatology 2016;63:787–798.

[43] **Pirazzi C**, **Adiels M**, Burza MA, Mancina RM, Levin M, Stahlman M, et al. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and in vitro. J Hepatol 2012;57:1276–1282.

[44] Yki-Järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2014;2:901–910.

[45] Yki-Järvinen H, Luukkonen PK. Heterogeneity of non-alcoholic fatty liver disease. Liver Int 2015;35:2498–2500.

[46] Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? Eur Heart J 2012;33:1190–1200.

[47] Al Knawy B, Shiffman M. Percutaneous liver biopsy in clinical practice. Liver Int 2007;27:1166–1173.

[48] Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology 2005;128:1898–1906.

[49] AlShaalan R, Aljiffry M, Al-Busafi S, Metrakos P, Hassanain M. Nonalcoholic fatty liver disease: Noninvasive methods of diagnosing hepatic steatosis. Saudi J Gastroenterol 2015;21:64–70.

[50] Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002;123:745–750.

[51] **Hernaez R**, **Lazo M**, Bonekamp S, Kamel I, Brancati FL, Guallar E, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;54:1082–1090.