Research Article

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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.

Keywords: Longitudinal study; Hepatic steatosis; Risk factors; Population study.
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Abbreviations: SNP, single nucleotide polymorphism; *PNPLA3*, patatin-like phospholipase domain-containing 3; BMI, body mass index; CRP, C-reactive protein; OR, odds ratios.

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 consent, 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 $\geqslant$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 <5.0×10^{-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