Association of *MBOAT7* gene variant with plasma ALT levels in children: the PANIC study

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BACKGROUND: We studied for the first time among children differences in plasma alanine aminotransferase (ALT) among genotypes of the rs641738 polymorphism in the *MBOAT7* gene that has been associated with increased risk of nonalcoholic fatty liver disease among adults. We also investigated the associations of a genetic risk score combining information from the *MBOAT7*, *PNPLA3*, and *TM6SF2* polymorphisms with plasma ALT.

METHODS: We performed a 2-y follow-up study in 467 Caucasian children aged 6–9 y, genotyped the *MBOAT7*, *PNPLA3*, and *TM6SF2* polymorphisms, calculated a genetic risk score from these polymorphisms (scored 0–3) and assessed plasma ALT.

RESULTS: Children carrying the T allele of the *MBOAT7* polymorphism had 7% higher plasma ALT at baseline (17.8 vs. 19.1 U/l, $P = 0.022$) and 10% higher plasma ALT at 2-y follow-up (18.0 vs. 19.7 U/l, $P = 0.022$) than the noncarriers. A higher genetic risk score was associated with higher plasma ALT at baseline (17.5, 18.5, 19.2, and 22.8 U/l, $P = 0.008$ for linear trend) and 2-y follow-up (18.2, 18.9, 18.9, and 32.8 U/l, $P = 0.017$ for linear trend).

CONCLUSION: Children carrying the T allele of the *MBOAT7* polymorphism had higher plasma ALT than the noncarriers. Children with the *MBOAT7*, *PNPLA3*, and *TM6SF2* variants had the highest plasma ALT.

Nonalcoholic fatty liver disease (NAFLD) is an important clinical and public health problem in developed countries (1,2). It is the most common liver disease not only in adults (2) but also in children (3) and affects up to 10% of all children and about 40% of obese children (1). There is accumulating evidence that certain gene variants predispose to the development of NAFLD in adults (3–5). The I148M variant of the *patatin-like phospholipase domain containing 3* (*PNPLA3*) gene has been associated with increased risk of NAFLD in adults (3) and also with increased liver fat content in children (6,7). The E167K variant of the *transmembrane 6 superfamily member 2* (*TM6SF2*) gene has been related to increased liver fat content

but with decreased plasma levels of triglycerides and total and low-density lipoprotein cholesterol in adults (4,8) and in children (9,10).

The rs641738 C > T variant in the membrane-bound O-acyltransferase domain-containing protein 7 (*MBOAT7*) gene was recently found to be associated with increased risk of developing NAFLD in adults (5). *MBOAT7*, also known as lysophosphatidylinositol acyltransferase 1, is an enzyme that is expressed in the liver (5) and is involved in the reacylation of phospholipids as part of the phospholipid remodeling pathway by specifically transferring polyunsaturated acyl-CoAs, such as arachidonoyl-CoA, to lysophosphatidylinositol and other lysophospholipids (11,12). The carriers of the T allele of the *MBOAT7* polymorphism were found to have reduced expression and synthesis of the *MBOAT7* protein in the liver and changes in plasma phosphatidylinositol species consistent with decreased *MBOAT7* function (5). These observations suggest that reduced enzymatic activity of *MBOAT7* explains the increased liver fat accumulation in the T allele carriers.

There is accumulating evidence that certain gene variants predispose to the development of NAFLD in adults (3–5) and in children (6,7,9,10) and that genetic risk scores may improve risk prediction for NAFLD beyond single risk variants in adults (13). There are no previous studies on the *MBOAT7* variant and its associations with liver fat content and other cardiometabolic risk factors among children. We therefore investigated the associations of the *MBOAT7* polymorphism with plasma alanine aminotransferase (ALT) and other cardiometabolic risk factors in a 2-y follow-up study in a population sample of Caucasian children 6–8 y of age. We also studied the associations of a genetic risk score combining information from the *MBOAT7*, *PNPLA3*, and *TM6SF2* polymorphisms with plasma ALT and other cardiometabolic risk factors among children.

RESULTS

Distributions of *MBOAT7*, *PNPLA3*, and *TM6SF2* polymorphisms

Of the 467 children examined at baseline, 157 (31%) were CC homozygotes, 222 (46%) were CT heterozygotes, and 78 (17%)

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were TT homozygotes for the *MBOAT7* polymorphism. Of the 395 children re-examined at 2-y follow-up, 130 (30%) were CC homozygotes, 197 (46%) were CT heterozygotes, and 68 (16%) were TT homozygotes. At baseline, there were 180 carriers (25 homozygotes), 287 noncarriers for the 148M allele of the *PNPLA3* polymorphism, 51 carriers (0 homozygotes), and 416 noncarriers for the 167K allele of the *TM6SF2* polymorphism. At 2-y follow-up, there were 163 carriers (21 homozygotes) and 232 noncarriers for the 148M allele of the *PNPLA3* polymorphism, 40 carriers (0 homozygotes) and 355 noncarriers for the 167K allele of the *TM6SF2* polymorphism.

Associations of *MBOAT7* Polymorphism with Plasma ALT and Other Cardiometabolic Risk Factors

At baseline, plasma ALT increased with the increasing number of the T alleles of the *MBOAT7* polymorphism after adjustment for age and sex (Table 1). Additional adjustment for body fat percentage slightly attenuated this association ($P = 0.063$ for linear trend). The carriers of the T allele of the *MBOAT7* polymorphism had 7% higher plasma ALT at baseline (Figure 1a) and 10% higher plasma ALT at 2-y follow-up (Figure 1b) than the noncarriers. Further adjustments had no effect on these differences.

At baseline, also body fat percentage and plasma high-sensitivity C-reactive protein (hsCRP) increased with the increasing number of the T alleles of the *MBOAT7* polymorphism after adjustment for age and sex (Table 1). Additional adjustment for body fat percentage slightly attenuated the association of the *MBOAT7* polymorphism with plasma hsCRP ($P = 0.074$ for linear trend). Moreover, further adjustment for plasma ALT slightly weakened the association of the *MBOAT7* polymorphism with body fat percentage ($P = 0.088$ for linear trend). Other adjustments had no effect on these associations. At 2-y follow-up, body fat percentage increased with the increasing number of the T alleles of the *MBOAT7* polymorphism adjusted for age and sex. Additional adjustment for plasma ALT attenuated the association between the *MBOAT7* polymorphism and body fat percentage ($P = 0.072$ for linear trend). Other adjustments had no effect on these associations.

Associations of Genetic Risk Score with Plasma ALT and other Cardiometabolic Risk Factors

A higher genetic risk score was associated with higher plasma ALT levels at baseline (Figure 2a) and at 2-y follow-up (Figure 2b) after adjustment for age and sex. Children carrying risk alleles for the *MBOAT7*, *PNPLA3*, and *TM6SF2* polymorphisms (score 3) had much higher plasma ALT levels at 2-y follow-up (Figure 2b) than children carrying fewer risk alleles (score 0–2). Children carrying all three risk alleles also had a larger increase in plasma ALT levels during the 2-y follow-up than children who carried fewer risk alleles adjusted for age and sex (+10.1 U/l vs. +0.2 – 0.5 U/l, $P = 0.003$ for difference between four groups). Additional adjustments had no effect on these associations. The genetic risk score was not associated with other cardiometabolic risk factors.

DISCUSSION

This is the first study on the associations of the rs641738 variant in the *MBOAT7* gene with plasma ALT and other cardiometabolic risk factors in children. Plasma ALT levels were higher among the carriers of the T allele of the *MBOAT7* polymorphism than among the noncarriers at baseline and at 2 y follow-up in our general population of children 6–8 y of age. Another potentially important finding of our study is that plasma ALT levels were highest among children carrying the risk alleles for the *MBOAT7*, *PNPLA3*, and *TM6SF2* polymorphisms, particularly at 2 y follow-up. Moreover, plasma ALT levels increased most during 2-y follow-up among children carrying all three risk alleles.

A recent study in adults showed that the carriers of the T allele of the *MBOAT7* polymorphism had increased risk for developing NAFLD (5). Our study in a general population of children provides new information beyond these findings among adults, because children carrying the T allele of the *MBOAT7* polymorphism had higher plasma ALT levels than the noncarriers. *MBOAT7*, also known as lysophosphatidylinositol acyltransferase 1, is an enzyme that is involved in the reacylation of phospholipids as part of the phospholipid remodeling pathway and specifically transfers polyunsaturated acyl-CoAs, such as arachidonoyl-CoA, to lysophosphatidylinositol and other lysophospholipids (11,12). *MBOAT7* has also been associated with anti-inflammatory processes by limiting the availability of free arachidonic acid in leukotriene B₄ synthesis in neutrophils (12). The recent study in adults showed that the *MBOAT7* gene is expressed in the liver and that the carriers of the T allele of the *MBOAT7* polymorphism have reduced expression and synthesis of the *MBOAT7* protein in the liver, changes in plasma phosphatidylinositol species consistent with decreased *MBOAT7* function and increased risk for developing NAFLD (5). These observations suggest that reduced enzymatic activity of *MBOAT7* explains increased liver fat accumulation in the T allele carriers.

A potentially important new finding of our study is that the *MBOAT7*, *PNPLA3*, and *TM6SF2* gene variants had an additive effect on plasma ALT levels at baseline and particularly at 2-y follow up as well as the increase in plasma ALT levels during 2-y follow-up among children. The combined effect of these three risk variants on plasma ALT levels is consistent with the results of the recent study among adults of their effect on liver fat accumulation (5). The additive effect of the *MBOAT7*, *PNPLA3*, and *TM6SF2* variants on plasma ALT levels suggests that a genetic risk score combining the information of these three gene variants may be a useful tool for screening children at increased risk for NAFLD.

We also found that body fat percentage at baseline and particularly at 2-y follow-up was higher among children who were homozygotes for the T allele of the *MBOAT7* polymorphism than among children with other genotypes. Furthermore, the association of the *MBOAT7* polymorphism with plasma ALT was partly explained by body fat percentage and vice versa. These results suggest that the *MBOAT7* protein might regulate

Table 1. Characteristics of children according to genotypes of *MBOAT7* polymorphism (rs641738) at baseline and at 2-y follow-up

	Baseline				2-y follow-up			
	CC <i>n</i> = 157	CT <i>n</i> = 232	TT <i>n</i> = 78	<i>P</i> -value	CC (<i>n</i> = 130)	CT (<i>n</i> = 197)	TT (<i>n</i> = 68)	<i>P</i> -value
Age (y)	7.6 (0.4)	7.7 (0.4)	7.6 (0.4)	0.992	9.7 (0.4)	9.8 (0.4)	9.7 (0.4)	0.823
Body height (cm)	128.4 (5.7)	128.7 (5.7)	128.8 (5.7)	0.577	140.2 (6.1)	140.4 (6.1)	140.7 (6.8)	0.578
Body weight (kg)	26.4 (4.2)	26.9 (5.1)	27.2 (4.9)	0.201	33.8 (6.2)	34.2 (7.6)	35.1 (7.3)	0.322
Body mass index –standard deviation score	–0.25 (0.97)	–0.19 (1.09)	–0.06 (1.14)	0.212	–0.15 (0.10)	–0.18 (1.1)	0.02 (1.1)	0.408
Body fat percentage (%)	19.0 (7.6)	19.6 (8.3)	21.4 (8.3)	0.030	22.9 (9.1)	22.8 (9.2)	25.8 (8.5)	0.037
Waist circumference (cm)	56.1 (4.6)	56.8 (6.0)	57.0 (5.7)	0.223	60.9 (6.3)	61.2 (7.7)	61.9 (6.8)	0.404
Serum insulin (mU/l)	4.61 (2.32)	4.46 (2.55)	4.59 (2.30)	0.726	6.01 (3.43)	5.74 (3.39)	6.59 (3.71)	0.325
Plasma glucose (mmol/l)	4.84 (0.43)	4.80 (0.39)	4.81 (0.36)	0.406	5.01 (0.32)	5.01 (0.62)	5.08 (0.42)	0.428
Plasma HDL cholesterol (mmol/l)	1.58 (0.31)	1.63 (0.32)	1.57 (0.29)	0.868	1.62 (0.34)	1.64 (0.33)	1.64 (0.31)	0.642
Plasma triglycerides (mmol/l)	0.62 (0.27)	0.57 (0.23)	0.61 (0.26)	0.348	0.64 (0.26)	0.62 (0.32)	0.61 (0.29)	0.240
Plasma alanine aminotransferase (U/l)	17.8 (5.4)	18.9 (6.0)	19.4 (7.9)	0.034	18.0 (8.5)	20.1 (10.9)	18.5 (6.4)	0.173
Plasma hsCRP (mg/l)	1.01 (0.08)	1.01 (0.11)	1.05 (0.22)	0.022	0.99 (2.57)	1.14 (3.92)	0.82 (1.06)	0.568

The *P*-values are from linear regression models adjusted for age and sex. The *P*-values of < 0.05 are in bold font. HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein. The boundary for statistical significance after Bonferroni correction is 0.004.

Figure 1. Means (95% confidence intervals) of plasma levels of alanine aminotransferase (ALT) in the noncarriers and carriers of the *MBOAT7* polymorphism adjusted for age and sex. Plasma ALT levels in the 157 noncarriers and 310 carriers at baseline (a) and in the 130 noncarriers and 265 carriers at 2 y follow up (b). * *P*=0.022

not only liver fat accumulation but also whole body adiposity. Another reason for these associations could be that increased whole body fat content increases liver fat accumulation and liver fat is part of total body fat percentage. These observations should be confirmed in other studies among children and adults, also more research is needed on the biological mechanisms for the effect of the *MBOAT7* variant in whole body fat accumulation. We also found that the TT homozygotes had slightly higher plasma levels of hsCRP, which is a protein secreted by the liver and an indicator of systemic low-grade inflammation, than children with other genotypes at baseline. However, this difference disappeared at 2-y follow-up.

The strength of this study include the population sample of girls and boys followed for 2 y, the comprehensive and valid measures of cardiometabolic risk factors and the opportunity to control for a number of possible confounding factors in the statistical analyses. The major weakness of our study is, liver fat content was not measured directly by magnetic resonance imaging or other imaging techniques. Another weakness is the limited statistical power to compare plasma ALT levels among the three genotypes of the *MBOAT7* polymorphism. Therefore,

we primarily compared plasma ALT levels at baseline and at 2-y follow-up in the carriers and noncarriers of the T allele of the *MBOAT7* polymorphism, and made the main conclusions of the findings based on these results. Also due to the limited statistical power, the results were not statistically significant after Bonferroni correction for multiple testing.

This study provides the first evidence that children carrying the T allele of the rs641738 polymorphism in the *MBOAT7* gene have higher plasma ALT levels than the noncarriers and that children with the combination of the *MBOAT7*, *PNPLA3*, and *TM6SF2* variants have the highest plasma ALT levels. These findings are consistent with the results of the recent study among adults, but should be confirmed in other studies among children and adults.

METHODS

Study Design and Study Population

The present analysis is based on the data of the Physical Activity and Nutrition in Children (PANIC) Study, which is an ongoing physical activity and diet intervention study in a population sample of children from the city of Kuopio, Finland. Altogether 736 children 6–9 y of age who started the first grade in primary schools

Figure 2. Means (95% confidence intervals) of plasma levels of alanine aminotransferase (ALT) according to the genetic risk score from the *MBOAT7*, *PNPLA3*, and *TM6SF2* polymorphisms (scores 0–3) adjusted for age and sex. At baseline (score 0, $n = 85$; score 1, $n = 225$; score 2, $n = 145$; and score 3, $n = 12$) (a) and at 2-y follow-up (score 0, $n = 72$; score 1, $n = 189$; score 2, $n = 123$; and score 3, $n = 11$) (b). * $P = 0.008$ for linear trend and ** $P = 0.017$ for linear trend.

of Kuopio in 2007–2009 were invited to participate in the baseline study in these years. Of the 736 invited children, 512 (70%) participated in the baseline study. Of these 512 children, 440 (86%) took part in the 2-y follow-up study. Data on main variables used in the baseline analyses were available for 467 children (222 girls and 245 boys) and those used in the 2-y follow-up analyses were available for 395 children (191 girls and 204 boys). These children were included in this study sample. The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo. Both children and their parents gave their written informed consent.

Genotyping

Genomic DNA was isolated from the blood mononuclear cells using the QIAamp DNA Blood kit (Qiagen, Hilden, Germany). The rs641738 polymorphism in the *MBOAT7* gene was genotyped in the Center for Inherited Disease Research, Johns Hopkins University, Baltimore, MD (<http://www.cidr.jhmi.edu/>), using the Custom Infinium Chemistry, Array-Cardio-Metabo_Chip_11395247_A (Illumina, San Diego, CA). The rs738409 polymorphism of the *PNPLA3* gene was genotyped using an allele-specific polymerase chain reaction (PCR) assay and a TaqMan probe (Applied Biosystems, Foster City, CA) according to the manufacturers' protocols. The E167K (rs58542926) polymorphism of the *TM6SF2* gene was genotyped at the Institute for Molecular Medicine Finland (FIMM) using the Infinium HumanCoreExome BeadChip (Illumina). The genotypes were determined using the BeadStudio and GenomeStudio softwares (Illumina). The final quality control was done using the PLINK software, Version 1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>) (14). The genotype distributions of the *MBOAT7*, *PNPLA3*, and *TM6SF2* gene polymorphisms were within the Hardy-Weinberg equilibrium.

Assessment of Cardiometabolic Risk Factors

After fasting of 12 hours blood samples were taken. A kinetic method according to the International Federation of Clinical Chemistry was used to analyze the plasma activity of ALT (Roche Diagnostics, Mannheim, Germany). A colorimetric enzymatic assay was used to analyze plasma concentration of triglycerides (Roche Diagnostics). Homogeneous enzymatic colorimetric assays were used to analyze plasma concentration of high-density lipoprotein cholesterol (Roche Diagnostics). A hexokinase method was used to analyze plasma glucose concentration (Roche Diagnostics). Serum insulin concentration was analyzed using an electrochemiluminescence immunoassay with the sandwich principle (Roche Diagnostics). Plasma high-sensitivity C-reactive protein was analyzed using enhanced immunoturbidimetric assay with the CRP (Latex) High Sensitive Assay reagent (Roche Diagnostics), the limit of detection being 0.15–0.20 mg/l.

Body weight was assessed twice after overnight fasting, bladder emptied and standing in light underwear by a calibrated InBody 720 bioelectrical impedance device (Biospace, Seoul, Korea) to accuracy of 0.1 kg. The mean of these two values of body weight was used for the analyses. Body height was assessed three times in the Frankfurt plane without shoes by a wall-mounted stadiometer to accuracy of 0.1 cm. The mean of the nearest two values of body height was used for the analyses. BMI – SD score was computed by the Finnish references (15). Waist circumference was assessed three times after expiration at mid-distance between the bottom of the rib cage and the top of the iliac crest with an unstretchable measuring tape to accuracy of 0.1 cm. The mean of the nearest two values was used for the analyses. Body fat percentage was assessed bladder emptied and lying in light clothing with all metal objects removed by the Lunar dual-energy X-ray absorptiometry device (Lunar Prodigy Advance, GE Medical Systems, Madison, WI). Pubertal stage was assessed by Tanner criteria (16).

Physical activity and sedentary behavior were assessed by the Actiheart heart rate and movement sensor (17). Dietary intake was assessed by food records of four consecutive days that consisted of two weekdays and two weekend days or three weekdays and one weekend day (18). The intakes of carbohydrates, sucrose and total, saturated, monounsaturated, and polyunsaturated fat as percentages of energy intake were analyzed using the Micro Nutrica dietary analysis software, Version 2.5 (Social Insurance Institution of Finland, Turku, Finland).

Statistical Methods

Statistical analyses were performed using the IBM SPSS Statistics software, Version 21 (IBM, Armonk, NY). Before statistical analyses, continuous variables with skewed distributions were log-transformed or square root-transformed. Linear trends for the associations of the *MBOAT7* polymorphism with plasma ALT and other cardiometabolic risk factors at baseline and at 2-y follow-up were analyzed using Linear Regression adjusted for age and sex. Differences in plasma ALT and other cardiometabolic risk factors between the carriers and noncarriers of the T allele of the *MBOAT7* polymorphism at baseline and at 2-y follow-up were analyzed by General Linear Model adjusted for age and sex. These data were additionally adjusted for the PANIC study group (intervention group vs. control group), the *PNPLA3* and *TM6SF2* polymorphisms, clinical puberty, physical activity, sedentary behavior, the dietary intakes of carbohydrates, sucrose, total saturated, monounsaturated, and polyunsaturated fat as percentages of energy intake and cardiometabolic risk factors, except the variable of interest, at baseline and the changes of these variables during 2-y follow-up. A genetic risk score for liver fat content was computed by giving a value 1 for carrying 1–2 risk alleles (no risk allele = 0, 1–2 risk alleles = 1) for the *MBOAT7*, *PNPLA3*, and *TM6SF2* polymorphisms, resulting in a range of 0–3 for the score. The associations of the genetic risk score with plasma ALT and other cardiometabolic risk factors were analyzed using Linear Regression, and General Linear Model adjusted for age and sex. These data were additionally adjusted using the same principle as explained above. Associations with a P -value of < 0.05 were considered statistically significant. We also used the Bonferroni correction for multiple testing. The threshold of statistical significance with the Bonferroni correction was 0.004 computed as the P -value of 0.05 divided by the number of variables used.

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