nature publishing group

Associations of TM6SF2 167K allele with liver enzymes and lipid profile in children: the PANIC Study

Anna Viitasalo[1], Jussi Pihlajamäki[2,3], Jussi Paananen[1,4], Mustafa Atalay[1], Virpi Lindi[1] and Timo A Lakka[1,5,6]

BACKGROUND: The 167K allele in the *TM6SF2* gene has been suggested to protect against cardiovascular disease at the cost of developing nonalcoholic fatty liver disease in adults.

METHODS: We performed a cross-sectional study in a population sample of 462 Caucasian children aged 6–9 y, genotyped the polymorphism using HumanCoreExome BeadChip, and assessed several cardiometabolic risk factors.

RESULTS: The 51 (11%) carriers of the 167K allele had higher plasma alanine aminotransferase (ALT) (20.8 vs. 18.4 U/l, $P = 0.011$) but lower plasma triglycerides (0.54 vs. 0.61 mmol/l, $P = 0.024$), total cholesterol (4.08 vs. 4.30 mmol/l, $P = 0.016$), and low-density lipoprotein (LDL) cholesterol (2.22 vs. 2.38 mmol/l, $P = 0.012$) than the 411 noncarriers. In factor analysis, the first factor was heavily loaded by plasma ALT (factor loading 0.63), triglycerides (−0.82), LDL cholesterol (−0.71), and waist circumference (0.61) in the carriers but not in the noncarriers.

CONCLUSIONS: The carriers of the 167K allele have higher plasma ALT but lower plasma triglycerides and total and LDL cholesterol than the noncarriers already in childhood.

Nonalcoholic fatty liver disease (NAFLD), recognized as the hepatic manifestation of the metabolic syndrome (1), is the most common liver disease not only in adults (2) but also in children (3). Increased liver fat content is typically associated with the overproduction of triglyceride-rich very-low-density lipoprotein particles from the liver due to the reduced insulin-induced suppression of very-low-density lipoprotein production that leads to hypertriglyceridemia and decreased plasma levels of high-density lipoprotein (HDL) cholesterol (1). NAFLD has been related to increased cardiovascular morbidity and mortality that has been suggested to be partly explained by hypertriglyceridemia and decreased plasma HDL cholesterol levels (2).

A recent exome-wide association study showed that adults with a cytosine-to-thymine substitution which replaces glutamate with lysine at residue 167 (E167K polymorphism) in the *TM6SF2* gene had increased hepatic triglyceride content and increased plasma levels of alanine aminotransferase (ALT) but decreased plasma levels of triglycerides and low-density lipoprotein (LDL) cholesterol (4). The carriers of the 167K allele were found to have a loss of function of the TM6SF2 that reduces the ability of the liver to export triglycerides in very-low-density lipoprotein particles to circulation that explains the increased liver fat content and the decreased plasma levels of triglycerides among the carriers (5). Consistent with these findings, other studies in adults have shown that the carriers of the 167K allele are more susceptible to NAFLD and liver fibrosis (6–9) but have a lower risk of developing cardiovascular disease that is partly explained by lower plasma total cholesterol levels among the carriers (7,10).

A recent study in obese children provided further evidence for the finding in adults that the carriers of the 167K allele in the *TM6SF2* gene have a higher liver fat content and higher plasma ALT levels but lower plasma levels of triglycerides and total and LDL cholesterol than the noncarriers (11). However, there are no studies in population samples of children most of whom have a normal body weight that would have compared plasma levels of liver enzymes, lipids, lipoproteins and other cardiometabolic risk factors, and the clustering of cardiometabolic risk factors in the carriers and noncarriers of the 167K allele. We therefore investigated these issues in a population sample of Caucasian children 6–9 y of age.

RESULTS

Differences in Cardiometabolic Risk Factors Between the Carriers and Noncarriers of the 167K Allele of the TM6SF2 Gene

Of all 462 children, 411 (89%) were 167E homozygotes, 51 (11%) were E167K heterozygotes and none was 167K homozygote. The 51 carriers of the I67K allele had higher plasma ALT levels but lower plasma levels of triglycerides, total cholesterol, and LDL cholesterol than the 411 noncarriers (**Table 1**). The carriers had 13% higher plasma ALT levels but 11% lower plasma triglyceride levels, 5% lower plasma total cholesterol levels, and 7% lower plasma LDL cholesterol levels than the noncarriers after adjustment for age and sex (**Figure 1**). Further adjustments with the patatin-like phospholipase domain containing 3 gene, diseases and medications that could

[1]Department of Physiology, Institute of Biomedicine, University of Eastern Finland, Kuopio Campus, Kuopio, Finland; [2]Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland; [3]Department of Clinical Nutrition and Obesity Center, Kuopio University Hospital, Kuopio, Finland; [4]Institute of Biomedicine, Bioinformatics Center, University of Eastern Finland, Kuopio, Finland; [5]Kuopio Research Institute of Exercise Medicine, Kuopio, Finland; [6]Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland. Correspondence: Anna Viitasalo (anna.viitasalo@uef.fi)

Received 4 August 2015; accepted 28 October 2015; advance online publication 17 February 2016. doi:10.1038/pr.2016.3

affect liver metabolism, clinical puberty, body fat percentage, physical activity, sedentary behavior, and dietary intakes of carbohydrates, sucrose and total, saturated, monounsaturated and polyunsaturated fat as percentages of energy intake had no effect on these differences (data not shown). There were no differences in other cardiometabolic risk factors between the carriers and the noncarriers (Table 1).

Table 1. Cardiometabolic risk factors in the carriers and noncarriers of the 167K allele of the *TM6SF2* gene

	Carriers of 167K allele ($n = 51$)	Noncarriers of 167K allele ($n = 411$)	*P* value*
Age (years)	7.6 (0.3)	7.6 (0.4)	0.392
Body height (cm)	128.9 (6.8)	128.6 (5.5)	0.746
Body weight (kg)	27.1 (5.5)	26.7 (4.7)	0.632
BMI–SDS	−0.14 (1.03)	−0.20 (1.07)	0.749
Waist circumference (cm)	56.7 (6.0)	56.5 (5.5)	0.925
Serum insulin (mU/l)	4.12 (2.03)	4.58 (2.48)	0.254
Plasma glucose (mmol/l)	4.79 (0.37)	4.81 (0.38)	0.719
Plasma total cholesterol (mmol/l)	4.06 (0.61)	4.30 (0.63)	**0.012**
Plasma LDL cholesterol (mmol/l)	2.19 (0.51)	2.38 (0.52)	**0.009**
Plasma HDL cholesterol (mmol/l)	1.62 (0.32)	1.60 (0.31)	0.773
Plasma triglycerides (mmol/l)	0.53 (0.21)	0.61 (0.25)	**0.015**
Plasma alanine aminotransferase (U/l)	21.0 (8.5)	18.3 (5.8)	**0.005**
Plasma gamma-glutamyltransferase (U/l)	11.6 (2.6)	11.7 (2.7)	0.773

The values are mean (SD) from the independent samples *t*-test.
BMI–SDS, body mass index–standard deviation score; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
*The *P* values of <0.05 are bolded.

Correlations Between Cardiometabolic Risk Factors in the Carriers and Noncarriers of the 167K Allele of the TM6SF2 Gene

Waist circumference correlated positively with plasma ALT in the carriers and noncarriers of the 167K allele after adjustment for age and sex (Tables 2 and 3). Waist circumference correlated negatively with plasma triglycerides in the carriers but positively in the noncarriers ($P = 0.001$ for interaction). Plasma ALT also correlated negatively with plasma triglycerides in the carriers but not in the noncarriers ($P = 0.018$ for interaction). Waist circumference correlated negatively with plasma total cholesterol in the carriers but not in the noncarriers ($P = 0.038$ for interaction). Waist circumference also correlated negatively with plasma LDL cholesterol in the carriers but not in the noncarriers ($P = 0.036$ for interaction). Further adjustments with the patatin-like phospholipase domain containing 3 gene, diseases and medications that could affect liver metabolism, clinical puberty, body fat percentage, physical activity, sedentary behavior and dietary intakes of carbohydrates, sucrose and total, saturated, monounsaturated and polyunsaturated fat as percentages of energy intake had no effect on the magnitude of these interactions (data not shown).

Factor Analysis of Cardiometabolic Risk Factors in the Carriers and Noncarriers of the 167K Allele of the TM6SF2 Gene

The first-order factor analysis with the promax rotation yielded three factors in the carriers and noncarriers of the 167K allele (Table 4). These factors explained cumulatively 73.4% of the variance in the carriers and 63.5% of the variance in the noncarriers. Among the carriers, the first "Liver adiposity, low triglycerides, and low LDL cholesterol factor" explaining 28.6%

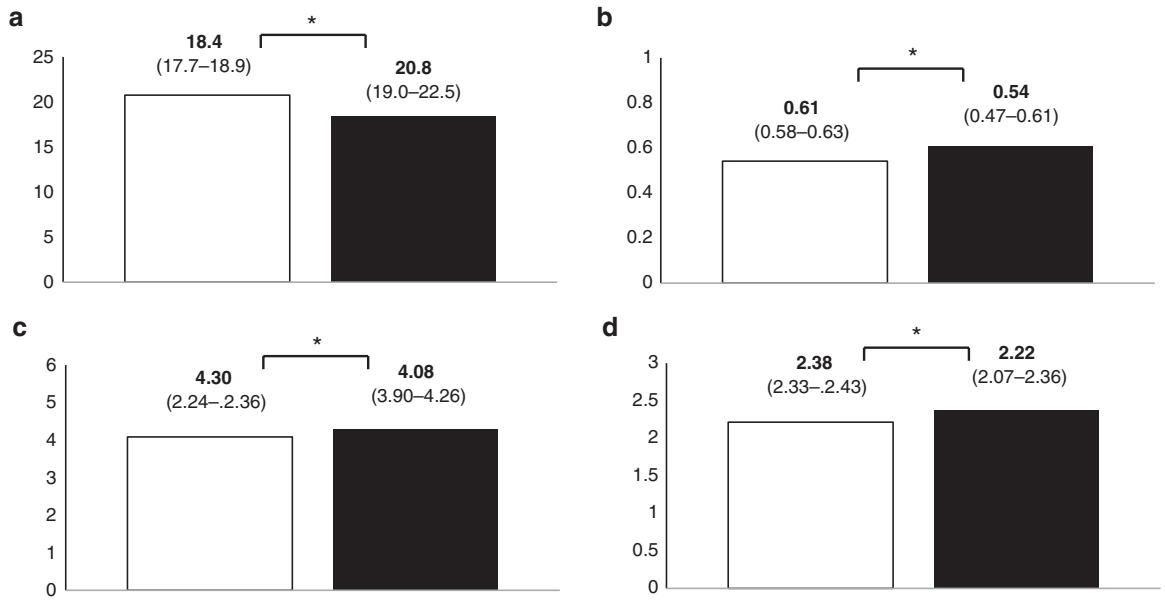

Figure 1. Differences in cardiometabolic risk factors between the non-carriers and carriers of the 167K allele of the *TM6SF2* gene. Means (95% CIs) of plasma levels of alanine aminotransferase (ALT) (**a**), triglycerides (**b**), total cholesterol (**c**) and low-density lipoprotein (LDL) cholesterol (**d**) in the 51 carriers (white bars) and 411 noncarriers (black bars) of the 167K allele of the *TM6SF2* gene adjusted for age and sex. **P* value < 0.05. CIs, confidence intervals.

of the variance was heavily loaded by ALT, inverse of triglycerides, inverse of LDL cholesterol, and waist circumference. The second "Insulin resistance factor" (26.8%) was heavily loaded by insulin, glucose, and waist circumference. The third "HDL cholesterol factor" (18.0%) was heavily loaded by inverse of HDL cholesterol, waist circumference, and insulin. Among the noncarriers, the first "Insulin resistance factor" (31.8%) was heavily loaded by insulin, glucose, and triglycerides. The second "Dyslipidemia factor" (17.1%) was heavily loaded by triglycerides, LDL cholesterol, inverse of HDL cholesterol, and insulin. The third "Liver adiposity factor" (14.7%) was heavily loaded by ALT and waist circumference.

Table 2. Correlations between cardiometabolic risk factors in the carriers of the 167K allele of the *TM6SF2* gene

	Waist	Insulin	Glucose	TG	Total chol	LDL chol	HDL chol
Waist							
Insulin	**0.463**						
Glucose	0.128	**0.586**					
TG	−0.251	0.213	**0.458**				
Total chol	**−0.295**	−0.107	0.115	**0.470**			
LDL chol	−0.226	−0.070	0.049	**0.550**	**0.794**		
HDL chol	−0.268	−0.178	0.045	−0.164	**0.365**	−0.208	
ALT	**0.307**	0.129	−0.036	**−0.356**	−0.101	−0.136	0.011

The values are partial correlation coefficients adjusted for age and sex. Statistically significant correlations are bolded.
ALT, alanine aminotransferase; HDL chol, HDL cholesterol; LDL chol, LDL cholesterol; TG, triglycerides; Total chol, total cholesterol; Waist, waist circumference.

Table 3. Correlations between cardiometabolic risk factors in the noncarriers of the 167K allele of the *TM6SF2* gene

	Waist	Insulin	Glucose	TG	Total chol	LDL chol	HDL chol
Waist							
Insulin	**0.380**						
Glucose	0.074	**0.569**					
TG	**0.180**	**0.437**	**0.279**				
Total chol	0.005	**0.138**	**0.123**	**0.233**			
LDL chol	0.078	**0.190**	**0.148**	**0.312**	**0.832**		
HDL chol	**−0.213**	**−0.183**	−0.075	**−0.308**	**0.369**	−0.088	
ALT	**0.223**	0.063	−0.078	−0.009	0.086	0.040	0.027

The values are partial correlation coefficients adjusted for age and sex. Statistically significant correlations are bolded.
ALT, alanine aminotransferase; HDL chol, HDL cholesterol; LDL chol, LDL cholesterol; TG, triglycerides; Total chol, total cholesterol; Waist, waist circumference.

DISCUSSION

This is the first study on the associations and interactions of the E167K polymorphism in the *TM6SF2* gene with plasma liver enzymes, lipids and lipoproteins, and other cardiometabolic risk factors in a population sample of children most of whom have a normal body weight (12). We found that plasma ALT levels were higher but plasma levels of triglycerides and total and LDL cholesterol were lower in the carriers of the 167K allele than in the noncarriers in a population sample of Caucasian children. We also observed that not only increased plasma ALT and decreased plasma triglycerides and total and LDL cholesterol but also increased waist circumference were associated with each other and were loaded in the same factor in the explorative factor analysis in the carriers of the 167K allele but not in the noncarriers.

Our findings are in agreement with the results of a few studies in adults (4,7,9,10,13) and one recent study among obese children (11) which showed that the carriers of the 167K allele of the *TM6SF2* gene have a higher liver fat content and higher plasma ALT levels and lower plasma levels of triglycerides and total and LDL cholesterol than the noncarriers. Other studies in adults have provided further evidence for these observations showing that the carriers of the 167K allele have a histologically more severe NAFLD than the noncarriers (6–9). The explanation for these findings is that the carriers of the 167K allele have a loss of function of the *TM6SF2* gene that reduces the ability of the liver to export triglycerides in very-low-density lipoprotein particles to circulation (5).

Table 4. Structure matrix for the factor analysis of cardiometabolic risk factors using the promax rotation in the carriers and noncarriers of the 167K allele of the *TM6SF2* gene

	Carriers of the 167K allele ($n = 51$)			Noncarriers of the 167K allele ($n = 411$)		
	Liver adiposity and low triglycerides and LDL cholesterol factor	Insulin resistance factor	HDL cholesterol factor	Insulin resistance factor	Dyslipidemia factor	Liver adiposity factor
Alanine aminotransferase	**0.63**	0.12	0.18	−0.07	−0.06	**0.79**
Triglycerides	**−0.82**	0.37	0.11	**0.50**	**0.76**	0.11
LDL cholesterol	**−0.71**	−0.03	0.27	0.23	**0.55**	0.02
HDL cholesterol	0.11	−0.07	**−0.86**	−0.06	**−0.75**	−0.15
Insulin	0.16	**0.86**	**0.46**	**0.86**	**0.44**	0.33
Glucose	−0.14	**0.83**	−0.14	**0.86**	0.18	−0.02
Waist circumference	**0.61**	**0.46**	**0.64**	0.31	0.31	**0.76**

R^2 for first, second, third factors, and cumulatively in the carriers (28.6, 26.8, 18.0, and 73.4%, respectively) and noncarriers (31.8, 17.1, 14.7, and 63.5%, respectively) of the 167K allele.
Heavily loaded correlation coefficients (≥0.40) are bolded.
LDL, low-density lipoprotein; HDL, high-density lipoprotein.

A novel finding of our study is that increased plasma ALT and decreased plasma triglycerides and total and LDL cholesterol but also increased waist circumference clustered in the carriers of the 167K allele but not in the noncarriers. This observation suggests that the typical dyslipidemia associated with the metabolic syndrome, including hypertriglyceridemia and decreased plasma HDL cholesterol, is replaced by decreased plasma triglycerides and LDL cholesterol that are accompanied by increased plasma ALT and waist circumference in the carriers of the 167K allele. However, other core features of the metabolic syndrome, including insulin resistance and abdominal adiposity, persisted both in the carriers and in the noncarriers. Another new observation is that waist circumference was inversely associated with triglycerides and total and LDL cholesterol in the carriers but not in the noncarriers, although it was directly related to plasma ALT both in the carriers and in the noncarriers. This finding suggests that abdominal adiposity increases the plasma triglyceride and total and LDL cholesterol lowering the effect of the 167K allele. The mechanism for this interaction between abdominal adiposity and the 167K allele remains unclear and warrants further studies.

Some other studies in adults have not confirmed the associations of carrying the 167K allele of the *TM6SF2* gene with increased plasma ALT and decreased plasma triglycerides and total and LDL cholesterol (9,14). The lack of these associations has been explained by the small sample sizes in these studies (14). The rather small sample size is also a weakness of our study. Although only 11% of the children carried the 167K allele, we observed that the carriers had statistically significantly higher plasma ALT levels and lower plasma levels of triglycerides and total and LDL cholesterol than the noncarriers. Moreover, we found that plasma ALT, triglycerides, total and LDL cholesterol, and waist circumference clustered in the carriers but not in the noncarriers. One reason for these clear findings despite the rather small sample size may be that the associations of the E167K polymorphism with the metabolic changes are less confounded by other cardiometabolic risk factors in children than in adults due to the shorter exposure to these risk factors. Another weakness of our study is that we could not assess liver fat content using MRI in our population study of children. On the other hand, a strength of our study is that we had the opportunity to investigate for the first time the associations and interactions of the E167K polymorphism with plasma liver enzymes, lipids and lipoproteins, adiposity, and other cardiometabolic risk factors in a population sample of children over 85% of whom had a normal body weight (12) and to control for a number of possible confounding factors.

The results of our study in a population sample of children suggest that higher plasma levels of ALT, lower plasma levels of triglycerides and LDL cholesterol and higher waist circumference clustered in the carriers but not in the noncarriers of the 167K allele of the *TM6SF2* gene. This information may be useful in the early identification of individuals at increased risk of NAFLD and cardiovascular disease.

METHODS

Study Design and Study Population

The present analyses are based on the baseline 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 of Kuopio in 2007–2009 were invited to participate in the baseline study in 2007–2009. Of the 736 invited children, 512 (70%) participated in the baseline study. Data on variables used in the analyses were available for 462 children (220 girls and 242 boys) who were included in the final 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 genotyping of the E167K (rs58542926) polymorphism of the *TM6SF2* gene was performed in the Institute for Molecular Medicine Finland (FIMM) using the Infinium HumanCoreExome BeadChip (Illumina, San Diego, CA). The genotypes were determined using the GenomeStudio software (Illumina). The final quality control was done using the PLINK, Version 1.07 software (http://pngu.mgh.harvard.edu/purcell/plink/) (15). The I148M (rs738409) polymorphism of the patatin-like phospholipase domain containing 3 gene was genotyped using an allele-specific PCR assay and a TaqMan probe (Applied Biosystems, Foster City, CA) according to the manufacturers' protocols. The genotype distributions of the E167K and I148M polymorphisms were within the Hardy–Weinberg equilibrium.

Assessment of Cardiometabolic Risk Factors

Blood samples were taken after fasting of 12 h. Kinetic methods according to the International Federation of Clinical Chemistry were used to analyze the plasma activities of ALT and gamma-glutamyl transferase (Roche Diagnostics, Mannheim, Germany). A colorimetric enzymatic assay was used to analyze plasma total cholesterol and triglycerides (Roche Diagnostics). Homogeneous enzymatic colorimetric assays were used to analyze plasma HDL and LDL cholesterol (Roche Diagnostics). A hexokinase method was used to analyze plasma glucose (Roche Diagnostics). Serum insulin was analyzed using an electrochemiluminescence immunoassay with the sandwich principle (Roche Diagnostics). Body weight was assessed twice after overnight fasting, with bladder emptying, and standing in light underwear by a calibrated InBody 720 bioelectrical impedance device (Biospace, Seoul, Korea) to an 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 an accuracy of 0.1 cm. The mean of the nearest two values of body height was used for the analyses. Body mass index–standard deviation score was computed by the Finnish references (16). 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 an accuracy of 0.1 cm. The mean of the nearest two values was used in statistical analyses. Body fat percentage was assessed with bladder emptying 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).

Other Assessments

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 food records were analyzed using the Micro Nutrica dietary analysis software, Version 2.5 (Social Insurance Institution of Finland, Turku, Finland). Pubertal stage was assessed by Tanner criteria.

Statistical Methods

Statistical analyses were performed with 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. Differences in cardiometabolic risk factors between the carriers and noncarriers of the 167K allele of the *TM6SF2* gene were compared by the *T*-test for independent samples. Differences in cardiometabolic risk factors between the carriers and the noncarriers were also compared by the General Linear Model adjusted for age and sex. Correlations between cardiometabolic risk factors in the carriers and noncarriers of the I67K allele of the *TM6SF2* gene were analyzed by computing partial correlation coefficients adjusted for age and sex. We also tested whether carrying the I67K allele modified the associations between cardiometabolic risk factors by General Linear Model adjusted for age and sex. Data were additionally adjusted for the 148M polymorphism of the patatin-like phospholipase domain containing 3 gene, diseases, and medications that could affect liver metabolism, clinical puberty, body fat percentage, physical activity, sedentary behavior and dietary intakes of carbohydrates, sucrose and total, saturated, monounsaturated, and polyunsaturated fat as percentages of energy intake. Associations with a P value of <0.05 were considered statistically significant. Moreover, we carried out factor analyses for cardiometabolic risk factors separately in the carriers and noncarriers of the 167K allele by entering ALT, triglycerides, LDL cholesterol, HDL cholesterol, insulin, and glucose as well as waist circumference in the models. The principal factor analysis was used for the extraction of the factors. Only factors with eigenvalues of >1.0 were retained in the analysis. The promax rotation that allows the variables to correlate with each other was used to assess possible underlying pathophysiological relationships. For the interpretation of the factors, variables having a correlation coefficient of ≥0.40 were considered to be heavily loaded.

ACKNOWLEDGMENTS

We thank the personnel of the Physical Activity and Nutrition in Children (PANIC) Study for performing this study.

STATEMENT OF FINANCIAL SUPPORT

This work has been financially supported by grants from the Ministry of Social Affairs and Health of Finland (Helsinki, Finland), the Ministry of Education and Culture of Finland (Helsinki, Finland), the University of Eastern Finland (Kuopio, Finland), the Finnish Innovation Fund Sitra (Helsinki, Finland), the Social Insurance Institution of Finland (Helsinki, Finland), the Finnish Cultural Foundation (Helsinki, Finland), the Juho Vainio Foundation (Helsinki, Finland), the Foundation for Paediatric Research (Helsinki, Finland), the Paavo Nurmi Foundation (Helsinki, Finland), the Diabetes Research Foundation (Tampere, Finland), the City of Kuopio (Kuopio, Finland), the Paolo Foundation (Espoo, Finland), the Research Committee of the Kuopio University Hospital Catchment Area for the State Research Funding and Kuopio University Hospital (EVO-funding number 5031343) (Kuopio, Finland).

Disclosure: There are no conflicts of interest.

REFERENCES

1. Kotronen A, Westerbacka J, Bergholm R, Pietiläinen KH, Yki-Järvinen H. Liver fat in the metabolic syndrome. J Clin Endocrinol Metab 2007;92:3490–7.
2. Azzam H, Malnick S. Non-alcoholic fatty liver disease - the heart of the matter. World J Hepatol 2015;7:1369–76.
3. Widhalm K, Ghods E. Nonalcoholic fatty liver disease: a challenge for pediatricians. Int J Obes (Lond) 2010;34:1451–67.
4. Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46:352–6.
5. Mahdessian H, Taxiarchis A, Popov S, et al. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. Proc Natl Acad Sci USA 2014;111:8913–8.
6. Liu YL, Reeves HL, Burt AD, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:4309.
7. Dongiovanni P, Petta S, Maglio C, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. Hepatology 2015;61:506–14.
8. Milano M, Aghemo A, Mancina RM, et al. TM6SF2 gene E167K variant impacts on steatosis and liver damage in chronic hepatitis C patients. Hepatology 2015;62:111–7.
9. Sookoian S, Castaño GO, Scian R, et al. Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. Hepatology 2015;61:515–25.
10. Holmen OL, Zhang H, Fan Y, et al. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. Nat Genet 2014;46:345–51.
11. Grandone A, Cozzolino D, Marzuillo P, et al. TM6SF2 Glu167Lys polymorphism is associated with low levels of LDL-cholesterol and increased liver injury in obese children. Pediatr Obes 2015; e-pub ahead of print 20 April 2015.
12. Eloranta AM, Lindi V, Schwab U, et al. Dietary factors associated with overweight and body adiposity in Finnish children aged 6-8 years: the PANIC Study. Int J Obes (Lond) 2012;36:950–5.
13. Dongiovanni P, Donati B, Fares R, et al. PNPLA3 I148M polymorphism and progressive liver disease. World J Gastroenterol 2013;19:6969–78.
14. Zhou Y, Llauradó G, Orešič M, Hyötyläinen T, Orho-Melander M, Yki-Järvinen H. Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. J Hepatol 2015;62:657–63.
15. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
16. Saari A, Sankilampi U, Hannila ML, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: length/height-for-age, weight-for-length/height, and body mass index-for-age. Ann Med 2011;43:235–48.
17. Brage S, Brage N, Franks PW, Ekelund U, Wareham NJ. Reliability and validity of the combined heart rate and movement sensor Actiheart. Eur J Clin Nutr 2005;59:561–70.
18. Eloranta AM, Lindi V, Schwab U, et al. Dietary factors and their associations with socioeconomic background in Finnish girls and boys 6-8 years of age: the PANIC Study. Eur J Clin Nutr 2011;65:1211–8.