Association of MTTP gene variants with pediatric NAFLD: A candidate-gene-based analysis of single nucleotide variations in obese children

Dongling Dai¹,²‡, Feiqiu Wen¹*, Shaoming Zhou¹*, Zhe Su¹‡, Guosheng Liu²‡, Mingbang Wang³,⁴‡, Jianli Zhou²‡, Fusheng He⁴‡

¹ Shenzhen Children’s Hospital, Shenzhen, China, ² First Affiliated Hospital of Jinan University, Guangzhou, China, ³ Key Laboratory of Birth Defects, Children’s Hospital of Fudan University, Shanghai, China, ⁴ Shenzhen Following Precision Medical Research Institute, Shenzhen, China

* These authors contributed equally to this work.
‡ These authors also contributed equally to this work.

fwen621@126.com (FW); zhousm15d@aliyun.com (SZ)

Abstract

Objective
We used targeted next-generation sequencing to investigate whether genetic variants of lipid metabolism-related genes are associated with increased susceptibility to nonalcoholic fatty liver disease (NAFLD) in obese children.

Methods
A cohort of 100 obese children aged 6 to 18 years were divided into NAFLD and non-NAFLD groups and subjected to hepatic ultrasound, anthropometric, and biochemical analyses. We evaluated the association of genetic variants with NAFLD susceptibility by investigating the single nucleotide polymorphisms in each of 36 lipid-metabolism-related genes. The panel genes were assembled for target region sequencing. Correlations between single nucleotide variations, biochemical markers, and clinical phenotypes were analyzed.

Results
97 variants in the 36 target genes per child were uncovered. Twenty-six variants in 16 genes were more prevalent in NAFLD subjects than in in-house controls. The mutation rate of MTTP rs2306986 and SLC6A2 rs3743788 was significantly higher in NAFLD subjects than in non-NAFLD subjects (OR: 3.879; P = 0.004; OR: 6.667, P = 0.005). Logistic regression analysis indicated the MTTP variant rs2306986 was an independent risk factor for NAFLD (OR: 23.468, P = 0.044).

Conclusions
The results of this study, examining a cohort of obese children, suggest that the genetic variation at MTTP rs2306986 was associated with higher susceptibility to NAFLD. This may
contribute to the altered lipid metabolism by disruption of assembly and secretion of lipoprotein, leading to reducing fat export from the involved hepatocytes.

Introduction

Nonalcoholic fatty liver disease (NAFLD) includes a range of liver diseases from simple fatty liver to nonalcoholic steatohepatitis (NASH), which can lead to fibrosis, cirrhosis, and hepatocellular carcinoma [1]. NAFLD is one of the most prevalent liver diseases among pediatric patients in developed countries owing to the increasing prevalence of obesity [2].

The precise pathogenesis of NAFLD remains poorly understood. Steatosis occurs when a rate of lipid influx or synthesis by hepatocytes exceeds the rate of export or catabolism [3]. The hepatic lipid metabolism pathways include hepatic de novo lipogenesis, lipolysis, transmembrane lipid flux, lipid oxidation, and peroxidation. An increasing number of studies identify genes that contribute to the high risk for developing pediatric NAFLD. Studies on the offspring of participants suggest a genetic predisposition to developing NAFLD [4], and heritability studies [5, 6] showed that nonalcoholic fatty liver disease is heritable. Moreover, familial aggregation studies [7] found that familial clustering of NAFLD was common. Genome-wide association studies (GWAS) of NAFLD subjects in Western countries identified several gene variants associated with NAFLD [8]. Gene expression studies reported that some genetic variants were associated with NAFLD [9].

Although insulin resistance, unhealthy diet, and sedentary lifestyle have been strongly associated with hepatic steatosis, accumulated evidence suggests that genetic background (specifically genetic polymorphisms) could be a critical factor for NAFLD predisposition in children [10, 11].

It is estimated that NAFLD affects 2.6–9.6% of pediatric patients and up to 38–53% of morbidly obese children worldwide [12]. The prevalence of NAFLD in children population is 2.1% and 68.2% among obese children in China [13]. Therefore, not every obese child develops NAFLD. We hypothesized that variants of genes in hepatic lipid metabolism pathways may contribute to increased susceptibility to pediatric NAFLD.

This study aimed to investigate the association of genetic variations with NAFLD susceptibility. We employed an approach of next-generation sequencing (NGS) and analyzed polymorphisms of 36 genes involved in hepatic lipid metabolism pathway in a cohort of children with or without NAFLD. The results of this study suggest that the genetic variation at MTTP rs2306986 was associated with higher susceptibility to pediatric NAFLD.

Methods

Study subjects

A total of 2236 children (of Han Chinese ethnicity) aged 6 to 18 years underwent regular physical examinations in 3 elementary and middle schools located in Shenzhen City, China. Among these children, 368 (16.5%) were considered obese according to the criteria adjusted with age and gender described by Cole et al [14].

100 of the 368 obese subjects were randomly selected and divided into a NAFLD group (group A) and a non-NAFLD group (group B). Individuals with a history of chronic liver disease (i.e., chronic hepatitis B and C, autoimmune disease, Wilson disease) as well as long-term drug consumption producing hepatic steatosis (i.e., corticosteroids), anemia, and hypothyroidism were excluded. The study protocol was approved by the Ethics Committee of
Shenzhen Children’s Hospital, and written informed consent was obtained from all participants’ parents.

**Childhood assessments and biochemical analyses**

Weight, height, waist circumference, and blood pressure of each participant were measured. The length tape measure and digital scale were accurate at 0.1 cm and 0.1 kg, respectively. BMI was calculated as body weight (kg)/height (m$^2$). Adjusted BMI = (BMI of study subject)—(median of age- and gender-specific standard BMI values).

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total serum bilirubin (TB), direct bilirubin (DB), fasting glucose, insulin, triglyceride (TG), total cholesterol (TC), high-density lipoproteins cholesterol (HDL-C), low-density lipoproteins cholesterol (LDL-C), apolipoprotein A1 (ApoA1), and apolipoprotein B (ApoB) were determined with routine biochemical assays. Insulin resistance was evaluated through the homeostasis model assessment of insulin resistance (HOMA-IR) and calculated using the formula fasting insulin (mmol/L) × fasting glucose (mmol/L)/22.5.

**Ultrasonography and magnetic resonance imaging (MRI)**

All participants underwent an ultrasonographic scan of the liver, performed by a single sonographer (Siemens Antares ultrasound machine with a CH 2- to 5-MHz convex probe). Then, a radiologist (specialized in liver imaging and blinded to the clinical and laboratory findings of the subjects) interpreted the ultrasound images. NAFLD was diagnosed using ultrasonographic scoring for liver steatosis and the findings of fatty infiltration (liver echotexture, echo penetration, and clarity of vessel structures) [15].

Subjects with the suggested NAFLD by ultrasonography were confirmed by MR imaging with a standard torso phased-array coil centered over the liver at 3-T MR imager (Signa Excite HD; GE Medical Systems, Milwaukee, WI; eight-channel coil). Two experienced radiologists reviewed images through Osirix and estimated the liver proton density fat fraction (PDFF), which is a measure of liver fat content [16].

**Targeted capture and next-generation sequencing**

Genomic DNA was extracted from 2 ml of ethylenediaminetetraacetic acid (EDTA) anticoagulated peripheral blood using a Qiagen DNA isolation kit (Qiagen, Valencia, CA), fragmented and used for sample library construction (Illumina Hiseq) according to the manufacturer’s instructions.

Briefly, 1 μg of genomic DNA in 100μl of TE was fragmented to a pool 150–250 bp by Bioruptor (Diagenode, Belgium), and then adapters (Invitrogen, USA) were ligated to both ends of the resultant fragments. The adapter-ligated templates were purified by the MagPure A3 XP beads (Magen, China). The purified DNA was amplified by ligation-mediated polymerase chain reaction, purified, and hybridized to lipid metabolism-related genes (LMRG) panel (iGeneTech, China) for enrichment. The target genes in LMRP panel including: TM6SF2, ACS1, GCKR, ACS3, ACACB, NR1I2, SREBF1, SREBF2, DGAT2, DGAT1, TNF, LPL, FASN, APOB, NCAN, FDF1, PEMT, FATP2 (SLC27A2), DLAT, SLC6A2, MTTP, PPP1R3B, ADIPOQ, CYP2E1, PAPAR, LEP, CPT1, UCP3, UCP1, PPARA, LIPE (HSL), SLC25A20 (CACT), LIPC (HL), PNPLA3, PNPLA2 (ATGL), CPT2. The hybridized fragments were bound to Streptavidin Dynabeads (Invitrogen, USA) and washed with proper stringent buffers (iGeneTech, China). The captured products were quantified with the Qubit dsDNA HS Assay Kit (Invitrogen, USA). Paired-end sequencing, which reads 150 bases from each end of the fragment for targeted libraries, was performed using Illumina HiSeq Xten and Illumina MiSeq instrumentation (Illumina, San Diego, CA).
Genetic variation detection and verification

Generated sequences in the clean reads were mapped the NCBI human reference genome (hg19/GRCh37) with Burrows-Wheeler Aligner, after using a quality filter (Trimmomatic) to remove reads containing sequencing adapters and low-quality reads. A low-quality read was defined as quality score less than 20 or a read shorter than 40 bases. Duplicates were marked using Picard (v1.54) software (http://picard.sourceforge.net/). GATK (Genome Analysis Toolkit) was used for calling SNPs and InDels. Annotation and classification for SNPs and InDels were obtained through ANNOVAR. The data was identified by dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), 1000 human genomes database (www.1000genomes.org/), and iGeneTech database (a database that is built on Whole Exome Sequencing based study of genetic risk for NAFLD, consisting of 2000 healthy Chinese people across China). Among the iGeneTech database, the 800 Han Chinese subjects were used as in-house controls. The inhouse controls were confirmed without NAFLD, metabolic disorders, diabetes mellitus, obesity, autoimmune hepatitis, dyslipidemia, or any family history of above diseases.

The variants were then selected using additional filter as following steps. First, the mutations in untranslated regions and splicing sites were removed. Then, the variants without functional prediction in at least one of the 4 algorithms (SIFT23, PolyPhen-2, Mutation Taster, and GERP++) that we used to investigate disease-causing potentials were discarded. Furthermore, the alterations that had more than 15% minor allele frequency (MAF) in one of the three databases of 1000 genomes, ESP6500si, and iGeneTech, or without MAF reported in the three databases were filtered. Finally, mutations without identification were excluded. The selected mutations were verified by Sanger sequencing.

Statistical analysis

SPSS v19.0 statistical software (StataCorp) was used for all the statistical analyses. Continuous variables were represented as the means ± SD. The two-tailed t-test was used for comparison of continuous variables across groups, while the Chi-squared (χ²) test and 1-factor ANOVA were used for comparisons of categorical variables. A P-value <0.05 was considered statistically significant. Potential associations between each single nucleotide variations (SNV) and NAFLD were tested using a χ² test for single SNP associations. The pair of the two SNVs was entered as a logistic regression model using Enter selection, and adjusted for the appropriate demographic variables and metabolic covariates.

Results and discussion

Subject characteristics

Thirty-nine (39%) of the 100 randomly selected obese participants were diagnosed with NAFLD. Age, sex, height, and systolic blood pressure (SBP) were not significantly different between the two groups (each P > 0.05). However, compared to the non-NAFLD group, NAFLD group subjects had higher waist circumference (WC), weight, BMI, and adjusted BMI values as well as higher levels of ALT, ALP, TG, TC, FFA, LDL-C, and ApoB (P < 0.05 for all parameters). However, there was no significant difference between the two groups in the levels of glucose, insulin, HOMA-IR, AST, TB, DB, HDL-C, and ApoA1 (P > 0.05 for all parameters). The demographic and biochemical characteristics of the study groups are described in Table 1.
Mutational analysis of genes

The variants that were not on target were excluded, resulting in 494 variants within the 36 target genes per subject (S1 Table). After completion of analysis steps by the functional filter described in Methods, 97 nonsynonymous exonic variants per patient were verified (S2 Table). All the mutations were scored as ‘damaging’ by at least 1 of the 4 algorithms (SIFT23, PolyPhen-2, Mutation Taster and GERP++). Mutation rates in the NAFLD subjects, non-NAFLD subjects and the in-house controls were compared, using Fisher’s Exact Test (S3 Table). Twenty-six SNVs were found to be enriched in the subjects with NAFLD when compared with in-house controls (all \( P < 0.05 \)) (S3 Table). The 26 SNPs were located in 16 genes; MTTP rs2306986 and SLC6A2 rs3743788 were significantly higher in subjects with NAFLD compared to non-NAFLD (OR: 3.879; \( P = 0.004 \); OR: 6.667, \( P = 0.005 \), respectively), see Table 2.

Table 1. Anthropometric and biochemical characters in NAFLD and non-NAFLD groups (of Han Chinese ethnicity).

| Variables            | NAFLD (N = 39) | Non-NAFLD (N = 61) | T    | P value |
|----------------------|----------------|--------------------|------|---------|
| Sex, M/F             | 19/20          | 37/24              | 1.376| 0.241   |
| Age at diagnosis (years) | 13.41 ± 2.26  | 13.54 ± 2.41      | 0.271| 0.787   |
| Systolic BP (mmHg)   | 123.15 ± 17.62 | 120.21 ± 12.00    | -0.981| 0.329   |
| Height (cm)          | 162.79 ± 12.61 | 162.07 ± 13.29    | -0.266| 0.791   |
| Weight (kg)          | 78.78 ± 16.84  | 68.27 ± 13.94     | -3.225| 0.002   |
| BMI (kg/m2)          | 29.64 ± 3.76   | 26.19 ± 2.63      | -5.343| <0.001  |
| Adjusted BMI (kg/m2) | 4.66 ± 0.59    | 0.71 ± 0.39       | -5.794| <0.001  |
| WC (cm)              | 98.07 ± 9.31   | 87.41 ± 8.37      | -5.380| <0.001  |
| TC (3.1–5.8 mmol/L)  | 4.58 ± 0.98    | 3.61 ± 0.71       | -5.715| <0.001  |
| TG (0.23–1.7 mmol/L) | 1.75 ± 0.74    | 1.08 ± 0.47       | -5.531| <0.001  |
| FFA (2.07–4.1 mg/dL) | 0.63 ± 0.20    | 0.53 ± 0.17       | -2.558| 0.013   |
| HDL-C (0.9–1.8 mmol/L)| 1.08 ± 0.28   | 1.06 ± 0.19       | -0.151| 0.709   |
| LDL-C (2.07–4.1 mmol/L)| 3.03 ± 0.68 | 2.45 ± 0.49       | -4.617| <0.001  |
| ApoA1 (1.05–2.05 g/L)| 1.23 ± 0.25    | 1.19 ± 0.23       | -0.744| 0.459   |
| ApoB (0.55–1.3 g/L)  | 1.04 ± 0.18    | 0.85 ± 0.24       | -4.549| <0.001  |
| ApoB/ApoA1           | 0.786 ± 0.037  | 0.779 ± 0.038     | -0.118| 0.906   |
| Glucose (3.1–5.6 mg/dL) | 5.42 ± 0.43  | 4.92 ± 0.15       | -1.104| 0.275   |
| Insulin (1.9–23 μU/mL)| 20.94 ± 2.90  | 31.65 ± 7.55      | 0.602 | 0.549   |
| HOMA-IR              | 4.79 ± 0.72    | 3.43 ± 0.68       | -1.364| 0.176   |
| TB (0.9–17.1 μmol/L) | 10.54 ± 0.84   | 9.57 ± 0.53       | -0.969| 0.336   |
| DB (0–6.08 μmol/L)   | 3.07 ± 0.48    | 2.38 ± 0.147      | -1.623| 0.108   |
| ALT (0–40 IU/L)      | 65.44 ± 10.83  | 17.93 ± 2.05      | -5.439| <0.001  |
| AST (0–40 IU/L)      | 40.77 ± 4.79   | 32.44 ± 6.31      | -1.562| 0.120   |
| ALP (40–500 IU/L)    | 246.90 ± 13.56 | 264.92 ± 7.85     | 2.921 | 0.005   |
| Lipid content        | 21.60 ± 3.19   | 11.25 ± 1.63      | -3.169| 0.002   |

WC, waist circumference; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TB, total serum bilirubin; DB, direct bilirubin; TG, triglyceride; TC, total cholesterol; HDLC, high-density lipoproteins cholesterol; LDL-C, low-density lipoproteins cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B.

https://doi.org/10.1371/journal.pone.0185396.t001

Mutational analysis of genes

Table 2. Comparison of mutation rate in MTTP rs2306986 and SLC6A2 rs3743788 between NAFLD group and non-NAFLD group (of Chinese Han ethnicity).

| SNV          | NAFLD (n = 39) | Non-NAFLD (n = 61) | \( \chi^2 \) value | OR    | P-value |
|--------------|----------------|--------------------|--------------------|-------|---------|
| MTTP rs2306986 | 19/20          | 12/49              | 9.331              | 3.879 | 0.004   |
| SLC6A2 rs3743788 | 10/29         | 3/58               | 7.294              | 6.667 | 0.005   |

https://doi.org/10.1371/journal.pone.0185396.t002
We further compared physical and biochemical findings between the subjects with and without variants of the two genes, and found that WC and the levels of ALT, TC, LDL, lipid content, and ApoB were significantly higher in the subjects with MTTP rs2306986 variant (P = 0.025, 0.001, 0.001, 0.005, 0.002, and <0.001, respectively), as shown in Table 3. The level of TG, TC, and ApoB was significantly higher in the subjects with SLC6A2 rs3743788 variant (p = 0.007, 0.029, and 0.003, respectively), as shown in Table 4. Binary logistic regression analysis indicated the MTTP rs2306986 was a risk factor for NAFLD (OR: 3666.537, P = 0.043), as shown in Table 5.

Discussion

This study revealed several interesting findings in phenotypes and genotypes of children with NAFLD. NAFLD was detected in 39% of the obese children in this study—lower than the 68.7% reported by Kodhelaj et al [17], 55.1% by Lin et al [18], and 42.9% by Duarte et al [19], but higher than the percentages reported by Pozzato et al (34.6%) [20] and Guijarro et al (30%) [21]. The difference in NAFLD may reflect the differences among the ethnic populations.

Table 3. The comparison of anthropometric and biochemical characteristics based on the presence of variation of MTTP rs2306986 (subjects of Han Chinese ethnicity).

| Variables          | MTTP rs2306986 | T-test or χ² test |
|--------------------|---------------|------------------|
|                    | A (N = 69)    | B (N = 31)       | T or F | P value |
| Sex (M/F)          | 25/44         | 9/22             | 0.494  | 0.482   |
| Age                | 13.23 ± 2.55  | 14.06 ± 1.67     | 1.662  | 0.100   |
| Height             | 160.98 ± 13.91| 165.33 ± 10.21   | 1.530  | 0.129   |
| Weight             | 71.15 ± 16.63 | 75.36 ± 14.24    | 1.216  | 0.227   |
| BMI                | 27.38 ± 3.63  | 27.86 ± 3.31     | 0.625  | 0.534   |
| Adjusted BMI       | 1.844 ± 0.46  | 3.15 ± 0.66      | 1.588  | 0.116   |
| WC                 | 90.04 ± 10.63 | 95.38 ± 8.02     | 2.284  | 0.025   |
| SBP                | 120.58 ± 14.06| 123.13 ± 15.52   | 0.807  | 0.422   |
| Glucose            | 5.14 ± 0.28   | 5.09 ± 0.17      | -0.111 | 0.912   |
| Insulin            | 31.96 ± 15.49 | 17.51 ± 2.81     | -0.626 | 0.533   |
| HOMA-IR            | 4.02 ± 0.68   | 3.85 ± 0.59      | -0.151 | 0.881   |
| TB                 | 9.90 ± 0.54   | 10.05 ± 0.87     | 0.145  | 0.885   |
| DB                 | 2.38 ± 0.15   | 3.25 ± 0.58      | 1.958  | 0.053   |
| ALT                | 25.48 ± 2.30  | 60.90 ± 13.91    | 3.585  | 0.001   |
| AST                | 24.35 ± 9.91  | 49.90 ± 18.78    | 2.023  | 0.046   |
| ALP                | 277.90 ± 80.95| 272.39 ± 81.19   | -0.315 | 0.754   |
| TG                 | 1.32 ± 0.08   | 1.38 ± 0.12      | 0.435  | 0.664   |
| Cholesterol        | 3.77 ± 0.87   | 4.46 ± 0.94      | 3.593  | 0.001   |
| FFA                | 0.56 ± 0.02   | 0.58 ± 0.03      | 0.277  | 0.783   |
| HDL                | 1.05 ± 0.03   | 1.12 ± 0.05      | 1.533  | 0.129   |
| LDL                | 2.55 ± 0.60   | 2.93 ± 0.62      | 2.894  | 0.005   |
| ApoA1              | 1.18 ± 0.24   | 1.25 ± 0.23      | 1.175  | 0.243   |
| ApoB               | 0.84 ± 0.03   | 1.11 ± 0.02      | 6.044  | 0.000   |
| ApoB/ApoA1         | 0.778 ± 0.035 | 0.792 ± 0.041    | -0.257 | 0.798   |
| Lipid content      | 12.82 ± 2.00  | 20.77 ± 2.80     | -0.125 | 0.026   |
| Diagnosis (N/n)    | 20/49         | 19/12            | 10.148 | 0.002   |

A, without variation; B, with variation; N, NAFLD; n, non-NAFLD; WC, waist circumference; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TB, total serum bilirubin; DB, direct bilirubin; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoproteins cholesterol; LDL-C, low-density lipoproteins cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B.

https://doi.org/10.1371/journal.pone.0185396.t003
We found that the BMI was significantly higher in the NAFLD group than in the non-NAFLD group, thus demonstrating that BMI may have significantly contributed to pediatric NAFLD development. This finding was consistent with the report that BMI was an independent risk factor for the formation of fatty liver [22].

On the other hand, we found no significant difference between the two groups in levels of insulin, glucose, and HOMA-IR. As previously reported, NAFLD was not associated with insulin secretion and insulin sensitivity in young obese children with strictly matched sex, age, pubertal status, and BMI [23]. These findings further supported our focus on hepatic lipid metabolism in this study [24].

Selecting candidate genes is challenging in the study of genetic polymorphism of NAFLD. To avoid arbitrariness, we selected the 36 genes involved in hepatic lipid metabolism in various ways including lipid synthesis, transmembrane lipid transport, lipolysis, and lipid oxidation.

### Table 4. The comparison of anthropometric and biochemical characters between subjects with and without the SLC6A2 rs3743788 variant (subjects of Han Chinese ethnicity).

| Variables         | SLC6A2 rs3743788 | T-test or χ² test |
|-------------------|------------------|------------------|
|                   | A (N = 87)       | B (N = 13)       | T or F     | P value  |
| Sex (M/F)         | 56/31            | 10/3             | 0.333      | 0.564    |
| Age               | 13.43 ± 2.42     | 13.84 ± 1.67     | -0.587     | 0.559    |
| Height            | 161.58 ± 13.41   | 167.26 ± 8.46    | -1.479     | 0.143    |
| Weight            | 71.36 ± 16.08    | 79.84 ± 13.43    | -2.062     | 0.054    |
| BMI               | 27.41 ± 3.61     | 28.26 ± 2.94     | -0.983     | 0.360    |
| Adjusted BMI      | 2.08 ± 0.41      | 3.33 ± 1.05      | -1.106     | 0.285    |
| WC                | 91.19 ± 10.21    | 94.64 ± 9.71     | -1.132     | 0.275    |
| SBP               | 121.19 ± 13.63   | 122.69 ± 19.92   | -0.263     | 0.797    |
| Glucose           | 5.13 ± 0.22      | 5.04 ± 0.33      | 0.224      | 0.825    |
| Insulin           | 28.33 ± 12.26    | 21.47 ± 6.06     | 0.502      | 0.617    |
| HOMA-IR           | 3.77 ± 0.52      | 5.23 ± 1.80      | -0.777     | 0.450    |
| TP                | 71.09 ± 4.14     | 70.38 ± 3.99     | 0.600      | 0.557    |
| ALB               | 42.21 ± 3.48     | 41.84 ± 2.87     | 0.433      | 0.670    |
| TB                | 10.12 ± 0.58     | 8.78 ± 1.00      | 1.197      | 0.246    |
| DB                | 2.70 ± 0.23      | 2.32 ± 0.43      | 0.780      | 0.445    |
| ALT               | 33.06 ± 4.83     | 59.23 ± 17.71    | -1.426     | 0.176    |
| AST               | 31.98 ± 6.78     | 34.23 ± 5.39     | -0.260     | 0.796    |
| ALP               | 279.32 ± 82.05   | 255.23 ± 69.74   | 1.134      | 0.272    |
| TG                | 1.27 ± 0.07      | 1.80 ± 0.18      | -2.793     | 0.013    |
| TC                | 3.91 ± 0.92      | 4.52 ± 0.98      | -2.108     | 0.052    |
| FFA               | 0.57 ± 0.02      | 0.56 ± 0.05      | 0.083      | 0.935    |
| HDL-C             | 1.08 ± 0.02      | 1.04 ± 0.05      | 0.514      | 0.608    |
| LDL-C             | 2.64 ± 0.63      | 2.89 ± 0.62      | -1.354     | 0.195    |
| ApoA1             | 1.21 ± 0.23      | 1.20 ± 0.26      | 0.119      | 0.906    |
| ApoB              | 0.89 ± 0.22      | 1.10 ± 0.26      | -2.747     | 0.015    |
| ApoB/ApoA1        | 0.787 ± 0.029    | 0.751 ± 0.069    | 0.477      | 0.640    |
| Lipid content     | 15.19 ± 1.85     | 15.89 ± 3.33     | -0.180     | 0.859    |
| Diagnosis (N/n)   | 29/58            | 10/3             | 9.033      | 0.003    |

A, without variation; B, with variation; N, NAFLD; n, non-NAFLD; WC, waist circumference; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TB, total serum bilirubin; DB, direct bilirubin; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoproteins cholesterol; LDL-C, low-density lipoproteins cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B.

https://doi.org/10.1371/journal.pone.0185396.t004
494 variants in the 36 genes per subject were detected in this cohort, and 97 of them were identified in each patient after functional filtration. Twenty-six variants in 16 genes were more prevalent in NAFLD subjects than in-house controls, but did not differ from non-NAFLD subjects.

Among the 16 genes, **ACACB**, **SREBF1**, **FASN**, **ACSS3**, and **DGAT2** involve lipid synthesis; **APOB**, **SLC27A2**, **MTTP**, **TNF**, and **SREBF1** participate in lipid influx and export from liver cells; **LIPE** (**HSL**), **PNPLA2** (**ATGL**), and **PNPLA3** are involved in lipolysis; and **ACACB**, **DLAT**, **PPARG**, **ADIPOQ**, and **CPT2** are involved in lipid oxidation. Thus, 16 genes could be associated with obesity in children. For instance, several of them have been identified in previous obesity studies including **ACACB**, **ACSS**, **ADIPOQ**, **DGAT2**, **HSL**, **FASN**, **PNPLA2**, **PNPLA3**, **PPAR-γ**, **SREBP1 SNP17** and **TNF** [25–37].

Furthermore, we found that the mutation rate of **MTTP** rs2306986 (c.294G>C, p.E98D) and **SLC6A2** rs3743788 (c.1646T>C, p.I549T) was significantly higher in subjects with NAFLD than that without NAFLD. Our results suggested that the two SNVs were associated with NAFLD in obese children. Triglycerides are either incorporated into VLDL particles for export or stored within the hepatocyte. Variations in lipid metabolism may lead to different rates of lipid accumulation in the hepatocyte.

The human microsomal triglyceride transfer protein (**MTTP** or **MTP**) carries lipid transfer function and is critical for the assembly and secretion of very-low-density lipoprotein (VLDL) to remove lipid from liver. Thus, changes in the liver lipid secretion efficiency (mediated by **MTTP**) can lead to hepatic steatosis [38]. Several lines of evidence have shown that **MTTP** polymorphisms may modulate the lipid homeostasis and may eventually lead to a high risk for NAFLD if such function is compromised because of genetic variation.

A large number of genetic polymorphisms in the **MTP** gene have been identified. In **MTTP**-knockout mice, there was a striking reduction in VLDL triglyceride accompanied by hepatic steatosis [39, 40]. The **MTP** -493G/T and GG polymorphism (rs1800591) have been implicated in the pathogenesis of NAFLD [41–44]. The GG genotype was associated with increased steatosis and histological NASH grade in NASH patients [45–48]. The 297H (rs2306985) variant increased the NAFLD risk by interaction with age, insulin resistance, and BMI [49]. The SNP -164 T/C (rs1800804) was associated with an increased risk of NAFLD in the Han Chinese population according to Peng et al [50].

### Table 5. Logistic regression for the two significant variants in subjects with and without NAFLD (subjects of Han Chinese ethnicity).

| Model term          | B     | S.E.  | Wald | OR    | 95% C.I. | P value |
|---------------------|-------|-------|------|-------|----------|---------|
| Constant            | -137.113 | 62.971 | 4.741 | 0.000 |          | 0.029   |
| Adjusted MBI        | 1.822 | 0.881 | 4.275 | 6.185 | 1.099, 34.794 | 0.039   |
| WC                  | 0.749 | 0.350 | 4.588 | 2.115 | 1.066, 4.196 | 0.032   |
| HOMAIR              | 0.321 | 0.230 | 1.952 | 1.378 | 0.879, 2.162 | 0.162   |
| TG                  | 4.300 | 2.602 | 2.732 | 73.736 | 0.450, 12090.635 | 0.098   |
| FFA                 | 26.608 | 13.050 | 4.157 | 3.595E11 | 2.802, 4.613E22 | 0.041   |
| TC                  | 4.578 | 2.430 | 3.551 | 97.335 | 0.832, 11383.280 | 0.060   |
| LDL-C               | 2.151 | 4.610 | 0.218 | 8.593 | 0.001, 72201.079 | 0.641   |
| ApoB                | 14.032 | 8.067 | 3.026 | 1241762.292 | 0.169, 9.130E12 | 0.082   |
| MTP rs2306986       | 8.207 | 5.605 | 2.144 | 3666.537 | 0.062, 2.165E8 | 0.043   |
| SLC6A2 rs3743788    | 0.608 | 2.298 | 0.070 | 1.837 | 0.020, 165.875 | 0.791   |
| SLC6A2 rs3743788* MTTP rs2306986 | -4.628 | 4.862 | 0.906 | 0.010 | 0.000, 134.467 | 0.341   |

BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; WC, waist circumference.

https://doi.org/10.1371/journal.pone.0185396.t005
These studies reasoned that common functional polymorphism in the human MTP gene may result in decreased protein production and inefficient regulation of hepatic lipid metabolism, thus contributing to the development of NAFLD [38, 51]. The mutation identified at rs2306986 in this study represents a new MTP variant and the impact on the function, as was predicted by PolyPhen-2, ranked as “possible damaging” with a score of 0.712 (sensitivity: 0.86; specificity: 0.92). This variant may alter gene expression to impair the function of MTP protein, contributing to the development of NAFLD.

Possible involvement of SLC6A2 in NAFLD pathogenesis has not been investigated. SLC6A2 gene encodes the norepinephrine transporter (NET), which is responsible for reuptake of norepinephrine into presynaptic nerve terminals and is a regulator of norepinephrine homeostasis. NET exerts a fine regulation of norepinephrine-mediated behavioral and physiological effects including mood, depression, feeding behavior, and cognition [52]. Individual variations in this gene were implicated in susceptibility to abnormal human behavior including depression and attention deficit [53]. Different combinations of T-182C and the G1287A polymorphisms of NET gene might increase morbidity risk in major depressive subpopulations [54]. In patients with major depressive disorder, there seemed to be a relationship between the volume of the dorsolateral prefrontal cortex and polymorphism of the SLC6A2 G1287A gene [54]. Furthermore, there was a correlation between the NET T1-82C polymorphism and the susceptibility to depression [55–57].

Depression was reported to be a risk factor for NAFLD [58]. The major depressive disorder was associated with more severe liver steatosis and poor treatment outcomes in patients with NAFLD [59]. In patients with NAFLD, depression was associated with more severe ballooning changes in hepatocytes [60]. Childhood obesity was associated with depression as reported by an Australia study [61]. Taken together, SLC6A2 polymorphisms may indirectly impact hepatic lipid metabolism by swinging psychological mood in obese children.

Moreover, the Reactome study (www.reactome.org) indicated that SLC6A2 (NET1) was associated with transport of hexose (glucose, fructose, metal ions), which correlated with coronary artery disease, height, glucose, and blood pressure according to the genome-wide association study. Furthermore, reactome reports that norepinephrine and epinephrine inhibit insulin secretion and they are the substrate of NET1; NET1 function is inversely regulated by insulin [62]. NAFLD is closely associated with insulin resistance and type 2 diabetes. The association of SLC6A2 polymorphisms with NAFLD may be mediated through insulin resistance.

There are limitations in this study. First, this cohort consisted of a relatively small sample and therefore our results need to be verified in multicenter-based large cohorts. Second, genetic variants detected in NAFLD should also be compared with well-matched normal healthy subjects, not just with in-house controls. Third, MTP appeared to be an important gene and its variants may have altered lipid metabolism, leading to NAFLD in obese children. However, we were not able to analyze MTP expression at mRNA and protein levels in this cohort. Finally, the ethnicity limitation was that only Han Chinese subjects were included in the present study and the genetic risk factor for NAFLD may differ among different ethnicities.

Conclusions

In this study, we analyzed genetic variants of 36 genes involved in lipid metabolism in 100 obese children. We found that the MTP rs2306986 (p < 0.05) and SLC6A2 rs3743788 (p < 0.05) variants were significantly associated with NAFLD. The presence of SNV (rs2306986) in the MTP gene was an independent risk factor for the susceptibility to NAFLD in obese children while the SLC6A2 polymorphism may exert indirect effect on the development of NAFLD. The identified
association of gene polymorphism and NAFLD may point to a more effective treatment strategy.

**Supporting information**

S1 Table. The 494 variants were detected among the 36 target genes per subject (of Han Chinese ethnicity) after using a quality filter (Trimmomatic) to remove reads containing sequencing adapters and low-quality reads.

(XLS)

S2 Table. Nonsynonymous SNVs in target region sequencing (subjects of Han Chinese ethnicity). A total of 97 nonsynonymous exonic variants per patient were verified within the 36 target genes per subject. All the mutations were scored as ‘damaging’ by at least 1 of the 4 algorithms (SIFT23, PolyPhen-2, Mutation Taster and GERP++).

(DOC)

S3 Table. The distributions of 26 SNVs and the comparison between NAFLD, non-ANFLD groups and in-house controls (of Han Chinese ethnicity). The mutations were compared using Fisher’s exact test. The 26 SNVs located in 16 genes were enriched in the NAFLD subjects compared to in-house controls (all P < 0.05).

(DOC)

**Acknowledgments**

The authors thank all children and their families for participating in and supporting this study. We also thank Dr Weiguo Cao (M.D, Radiologist, Radiology Department of Shenzhen Children’s Hospital) for technical assistance with the imaging.

**Author Contributions**

**Conceptualization:** Shaoming Zhou.

**Data curation:** Guosheng Liu, Jianli Zhou.

**Formal analysis:** Dongling Dai.

**Funding acquisition:** Dongling Dai.

**Investigation:** Zhe Su, Jianli Zhou.

**Methodology:** Dongling Dai, Zhe Su, Mingbang Wang, Fusheng He.

**Project administration:** Feiqiu Wen, Mingbang Wang, Fusheng He.

**Resources:** Jianli Zhou.

**Software:** Guosheng Liu.

**Supervision:** Feiqiu Wen.

**Writing – original draft:** Dongling Dai.

**Writing – review & editing:** Dongling Dai.

**References**

1. Day CP. Non-alcoholic fatty liver disease: a massive problem. Clinical medicine. 2011; 11(2):176–8. PMID: 21526706
2. Alisi A, Manco M, Vania A, Nobili V. Pediatric nonalcoholic fatty liver disease in 2009. The Journal of pediatrics. 2009; 155(4):69–74. https://doi.org/10.1016/j.ped.2009.06.014 PMID: 1977298

3. Bradbury MW, Berk PD. Lipid metabolism in hepatic steatosis. Clinics in liver disease. 2004; 8(3):639–71. x. https://doi.org/10.1016/j.clld.2004.04.005 PMID: 15331068

4. Loomba R, Hwang SJ, O'Donnell C, Ellison RC, Vasan RS, D'Agostino RB Sr., et al. Parental obesity and offspring serum alanine and aspartate aminotransferase levels: the Framingham heart study. Gastroenterology. 2008; 134(4):953–9. https://doi.org/10.1053/j.gastro.2008.01.037 PMID: 18395076

5. Brouwers MC, van Greevenbroek MM, Cantor RM. Heritability of nonalcoholic fatty liver disease. Gastroenterology. 2009; 137(4):1536. https://doi.org/10.1053/j.gastro.2009.03.065 PMID: 19717129

6. Schwimmer JB, Celedon MA, Lavine JE, Campbell N, Schork NJ, et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology. 2009; 136(5):1585–92. https://doi.org/10.1053/j.gastro.2009.01.050 PMID: 19208353

7. Abdelmalek MF, Liu C, Shuster J, Nelson DR, Asal NR. Familial aggregation of insulin resistance in first-degree relatives of patients with nonalcoholic fatty liver disease. Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association. 2006; 4(9):1162–9.

8. Chalasani N, Guo X, Loomba R, Goodarzi MO, Haritunians T, Kwon S, et al. Genome-wide association study identifies variants associated with histologic features of nonalcoholic fatty liver disease. Gastroenterology. 2010; 139(5):1567–76, 76 e1–6. https://doi.org/10.1053/j.gastro.2010.07.057 PMID: 20708005

9. Shang XR, Song JY, Liu FH, Ma J, Wang HJ. GWAS-Identified Common Variants With Nonalcoholic Fatty Liver Disease in Chinese Children. Journal of pediatric gastroenterology and nutrition. 2015; 60(5):669–74. https://doi.org/10.1016/j.jpeds.2015.05.043 PMID: 26028579

10. Daly AK, Ballestri S, Carulli L, Loria P, Day CP. Genetic determinants of susceptibility and severity in nonalcoholic fatty liver disease. Expert review of gastroenterology & hepatology. 2011; 5(2):253–63.

11. Valenti L, Alisi A, Galmozzi E, Bartuli A, Del Menico B, Alterio A, et al. I148M patatin-like phospholipase domain-containing gene variant and severity of pediatric nonalcoholic fatty liver disease. Hepatology. 2010; 52(4):1274–80. https://doi.org/10.1002/hep.23823 PMID: 20648474

12. Angulo P. GI epidemiology: nonalcoholic fatty liver disease. Alimentary pharmacology & therapeutics. 2007; 25(8):883–9.

13. Fan JG. Epidemiology of alcoholic and nonalcoholic fatty liver disease in China. Journal of gastroenterology and hepatology. 2013; 28 Suppl 1:11–7.

14. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatric obesity. 2012; 7(4):284–94. https://doi.org/10.1111/j.2047-6310.2012.00064.x PMID: 22715120

15. Lin YC, Chou SC, Huang PT, Chiou HY. Risk factors and predictors of non-alcoholic fatty liver disease in Taiwan. Annals of hepatology. 2011; 10(2):125–32. PMID: 21502673

16. Achmad E, Yokoo T, Hamilton G, Heba ER, Hooker JC, Changchien C, et al. Feasibility of and agreement between MR imaging and spectroscopic estimation of hepatic proton density fat fraction in children with known or suspected nonalcoholic fatty liver disease. Abdominal imaging. 2015; 40(8):3084–90. https://doi.org/10.1002/adi.23597 PMID: 26309261

17. Kodheja K, Resuli B, Petrela E, Malaj V, Jaze H. Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in Albanian overweight children. Minerva pediatrica. 2014; 66(1):23–30. PMID: 24608579

18. Lin YC, Chang PF, Yeh SJ, Liu K, Chen HC. Risk factors for liver steatosis in obese children and adolescents. Pediatrics and neonatology. 2010; 51(3):149–54. https://doi.org/10.1016/j.peds.2009.06.014 PMID: 20675238

19. Duarte MA, Silva GA. Hepatic steatosis in obese children and adolescents. Jornal de pediatria. 2011; 87(2):150–6. https://doi.org/10.2223/JPED.2065 PMID: 21503382

20. Pozzato C, Verduci E, Scaglioni S, Radaelli G, Salvioni M, Rovere A, et al. Liver fat change in obese children after a 1-year nutrition-behavior intervention. Journal of pediatric gastroenterology and nutrition. 2010; 51(3):331–5. https://doi.org/10.1016/j.jpeds.2009.05.028 PMID: 20562718

21. Guijarro de Armas MG, Monereo Megias S, Navea Aguilera C, Merino Viveros M, Vega Pinero MB. [Non-alcoholic fatty liver in children and adolescents with excess weight and obesity]. Medicina clinica. 2015; 144(2):55–8. https://doi.org/10.1016/j.medci.2014.02.018 PMID: 24768200

22. Hsiao PJ, Chen ZC, Hung WW, Yang YH, Lee MY, Huang JF, et al. Risk interaction of obesity, insulin resistance and hormone-sensitive lipase promoter polymorphisms (LIPE-60 C > G) in the development of fatty liver. BMC medical genetics. 2013; 14:54. https://doi.org/10.1186/1471-2350-14-54 PMID: 23688034
23. Bedogni G, Mari A, De Col A, Marazzi N, Tinibelli C, Manco M, et al. Nonalcoholic Fatty Liver Is Not Associated with the Relationship between Insulin Secretion and Insulin Sensitivity in Obese Children: Matched Case-Control Study. Childhood obesity. 2016; 12(6):426–31. https://doi.org/10.1089/chi.2016.0141 PMID: 27541280

24. Pacifico L, Nobili V, Anania C, Verdeccchia P, Chiesa C. Pediatric nonalcoholic fatty liver disease, metabolic syndrome and cardiovascular risk. World journal of gastroenterology. 2011; 17(26):3082–91. https://doi.org/10.3748/wjg.v17.i26.3082 PMID: 21912450

25. Eberle D, Clement K, Meyre D, Sahbatou M, Vaxillaire M, Le Gall A, et al. SREBF-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. Diabetes. 2004; 53(8):2153–7. PMID: 15277400

26. Friedel S, Reichwald K, Scherag A, Brumm H, Wermter AK, Fries HR, et al. Mutation screen and association studies in the diacylglycerol O-acyltransferase homolog 2 gene (DGAT2), a positional candidate gene for early onset obesity on chromosome 11q13. BMC genetics. 2007; 8:17. https://doi.org/10.1186/1471-2156-8-17 PMID: 17477860

27. Klannemark M, Orho M, Langin D, Laurell H, Holm C, Reynisdottir S, et al. The putative role of the hormone-sensitive lipase gene in the pathogenesis of Type II diabetes mellitus and abdominal obesity. Diabetologia. 1998; 41(12):1516–22. https://doi.org/10.1007/s001250051099 PMID: 9867220

28. Lu JF, Zhou Y, Huang GH, Jiang HX, Hu BL, Qin SY. Association of ADIPOQ polymorphisms with obesity and type 2 diabetes in French obese and diabetic cohorts. Diabetes. 2004; 53(8):2153–7. PMID: 15277400

29. Moreno-Navarrete JM, Botas P, Valdes S, Ortega FG, Delgado E, Vazquez-Martin A, et al. Val1483Ile in PPARgamma Pro12Ala and ACE ID polymorphisms are associated with obesity and type 2 diabetes in Asian Indians in North India. Molecular biology reports. 2013; 40(11):670–6. https://doi.org/10.1007/s11033-013-2738-5 PMID: 24078163

30. Passaro A, Dalla Nora E, Marcello C, Di Vece F, Morieri ML, Sanz JM, et al. PPARgamma Pro12Ala and ACE ID polymorphisms are associated with BMI and fat distribution, but not metabolic syndrome. Cardiovascular diabetology. 2011; 10:112. https://doi.org/10.1186/1475-2840-10-112 PMID: 22168210

31. Phillips CM, Goumidi L, Bertrais S, Field MR, Cupples LA, Ordovas JM, et al. ACC2 gene polymorphisms, metabolic syndrome, and gene-nutrient interactions with dietary fat. Journal of lipid research. 2010; 51(12):3500–7. https://doi.org/10.1194/jlr.M008474 PMID: 20855566

32. Riancho JA, Vazquez L, Garcia-Perez MA, Sainz J, Olmos JM, Hernandez JL, et al. Association of ACACB polymorphisms with obesity and diabetes. Molecular genetics and metabolism. 2011; 104(4):670–6. https://doi.org/10.1016/j.ymgme.2011.08.013 PMID: 21908218

33. Sharma M, Vikram NK, Misra A, Bhatt S, Tarique M, Parray HA, et al. Assessment of 11-beta hydroxysteroid dehydrogenase (11-betaHSD1) 4478T>G and tumor necrosis factor-alpha (TNF-alpha)-308G>A polymorphisms with obesity and insulin resistance in Asian Indians in North India. Molecular biology reports. 2013; 40(11):6261–70. https://doi.org/10.1007/s11033-013-2738-5 PMID: 24078163

34. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95(5):2498–502. PMID: 9482914

35. Watt KD, Dierkhising R, Fan C, Tillman H, Goldstein D, et al. Investigation of PNPLA3 and IL28B genotypes on obesity and diabetes after liver transplantation: insight into mechanisms of disease. American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2013; 13(9):2450–7.

36. Yu GI, Ha E, Park SH, Park JH, Jang HS, Bae JH, et al. Association of tumor necrosis factor-alpha (TNF-alpha) promoter polymorphisms with overweight/obesity in a Korean population. Inflammation research: official journal of the European Histamine Research Society [et al]. 2011; 60(12):1099–105.

37. Zegers D, Verrijken A, Beckers S, Francqu S, Van Camp JK, Aerts E, et al. Association study of PNPLA2 gene with histological parameters of NAFLD in an obese population. Clinics and research in hepatology and gastroenterology. 2016; 40(3):333–9. https://doi.org/10.1016/j.clinre.2015.09.001 PMID: 26500201

38. Pereira IV, Stefano JT, Oliveira CP. Microsomal triglyceride transfer protein and nonalcoholic fatty liver disease. Expert review of gastroenterology & hepatology. 2011; 5(2):245–51.

39. Day CP. Genetic and environmental susceptibility to non-alcoholic fatty liver disease. Digestive diseases. 2010; 28(1):255–60. https://doi.org/10.1159/000282098 PMID: 20460920

40. Raabe M, Veniant MM, Sullivan MA, Zlot CH, Bjorkgren J, Nielsen LB, et al. Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice. The Journal of clinical investigation. 1999; 103(9):1287–98. https://doi.org/10.1172/JCI6576 PMID: 10225972

41. EI-Koofy NM, EI-Karaky HM, Mandour IM, Anwar GM, EI-Raziky MS, EI-Hennawy AM. Genetic polymorphisms in non-alcoholic fatty liver disease in obese Egyptian children. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association. 2011; 17(4):265–70.
50. Peng XE, Wu YL, Lu QQ, Hu ZJ, Lin X. MTTP polymorphisms and susceptibility to non-alcoholic fatty liver disease in a Han Chinese population. Liver international: official journal of the International Association for the Study of the Liver. 2005; 25(3):2492–6. PMID: 16321277

51. Namikawa C, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, et al. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. Journal of hepatology. 2004; 40(5):781–6. https://doi.org/10.1016/j.jhep.2004.01.028 PMID: 15094225

52. Stan S, Lambert M, Delvin E, Paradis G, O’Loughlin J, Hanley JA, et al. Intestinal fatty acid binding protein and microsomal triglyceride transfer protein polymorphisms in French-Canadian youth. Journal of lipid research. 2012; 53(3):548–55. https://doi.org/10.1194/jlr.M020024 PMID: 22236406

53. Li RS, Yin RX, Lin WX, Yang DZ. [Relationship between the polymorphism of microsomal triglyceride transfer protein gene and the level of serum lipids in Guangxi Heiyi Zhuang population]. Zhonghua yi xue yichuan xue za zhi = Chinese journal of medical genetics. 2009; 26(4):388–92. PMID: 20017301

54. Siqueira ER, Oliveira CP, Correa-Giannella ML, Stefano JT, Cavaleiro AM, Fortes MA, et al. MTP-297H polymorphism reduced serum cholesterol but increased risk of non-alcoholic fatty liver disease-a cross-sectional study. BMC medical genetics. 2015; 16:93. https://doi.org/10.1186/s12881-015-0242-6 PMID: 26457295

55. Li L, Wang SJ, Shi K, Chen D, Jia H, Zhu J. Correlation between MTP -493G>T polymorphism and non-alcoholic fatty liver disease risk: a meta-analysis. Genetics and molecular research: GMR. 2014; 13(4):10150–61. https://doi.org/10.4238/2014.December.4.9 PMID: 25501226

56. Musso G, Gambino R, Cassader M. Lipoprotein metabolism mediates the association of MTP polymorphism with beta-cell dysfunction in healthy subjects and in non-diabetic normolipidemic patients with nonalcoholic steatohepatitis. The Journal of nutritional biochemistry. 2010; 21(8):834–40. https://doi.org/10.1016/j.jnutbio.2009.06.007 PMID: 19733470

57. Siqueira ER, Oliveira CP, Correa-Giannella ML, Stefano JT, Cavaleiro AM, Fortes MA, et al. MTP-297H polymorphism reduced serum cholesterol but increased risk of non-alcoholic fatty liver disease-a cross-sectional study. BMC medical genetics. 2015; 16:93. https://doi.org/10.1186/s12881-015-0242-6 PMID: 26457295

58. Bernstein RP, Benjamin J, Belmaker RH. Personality and polymorphisms of genes involved in aminergic neurotransmission. European journal of pharmacology. 2000; 410(2–3):205–14. PMID: 11134670

59. Wang Y, Sun N, Li S, Du Q, Xu Y, Liu Z, et al. A Genetic Susceptibility Mechanism for Major Depression: Combinations of polymorphisms Defined the Risk of Major Depression and Subpopulations. Medicine. 2015; 94(23):e778. https://doi.org/10.1016/j.mder.2015.01.028 PMID: 26061302

60. Min WJ, Ma XH, Li T, Zhang B, Sun XL. [Association of serotonin and norepinephrine transporter gene variants with pediatric NAFLD]. Zhonghua yi xue yichuan xue za zhi = Journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 2012; 45(1):72–7. https://doi.org/10.1590/S0100-879X2012007500160 PMID: 22147193

61. Min W, Li T, Ma X, Li Z, Yu T, Gao D, et al. Monoamine transporter gene polymorphisms affect susceptibility to non-alcoholic fatty liver disease in a Han Chinese population. Liver international: official journal of the International association for the Study of the Liver. 2014; 34(1):118–28.

62. Di Filippo M, Crehalet H, Samson-Bouma ME, Bonnet V, Aggerbeck LP, Rabes JP, et al. Molecular and functional analysis of two new MTTP gene mutations in an atypical case of abetalipoproteinemia. Journal of lipid research. 2005; 46(2):320–7. https://doi.org/10.1194/jlr.M400346-JLR200 PMID: 15547295

63. Bonisch H, Bruss M. The norepinephrine transporter in physiology and disease. Handbook of experimental pharmacology. 2006(175):485–524. PMID: 16722247

64. Ebstein RP, Benjamin J, Belmaker RH. Personality and polymorphisms of genes involved in aminergic neurotransmission. European journal of pharmacology. 2000; 410(2–3):205–14. PMID: 11134670

65. Wang Y, Sun N, Li S, Du Q, Xu Y, Liu Z, et al. A Genetic Susceptibility Mechanism for Major Depression: Combinations of polymorphisms Defined the Risk of Major Depression and Subpopulations. Medicine. 2015; 94(23):e778. https://doi.org/10.1016/j.mder.2015.01.028 PMID: 26061302

66. Min W, Li T, Ma X, Li Z, Yu T, Gao D, et al. Monoamine transporter gene polymorphisms affect susceptibility to depression and predict antidepressant response. Psychopharmacology. 2009; 205(3):409–17. https://doi.org/10.1007/s00213-009-1550-3 PMID: 19468717

67. Min WJ, Ma XH, Li T, Zhang B, Sun XL. [Association of serotonin and norepinephrine transporter gene variants with the susceptibility to depression]. Zhonghua yi xue yichuan xue za zhi = Chinese journal of medical genetics. 2009; 26(4):388–92. PMID: 20017301

68. Phillips JL, Batten LA, Tremblay P, Aldosary F, Du L, Blier P. Impact of monoamine-related gene polymorphisms on hippocampal volume in treatment-resistant depression. Acta neuropsychiatrica. 2015; 27(6):535–61. https://doi.org/10.1017/neu.2015.25 PMID: 25990886

69. Macavei B, Baban A, Dumitrascu DL. Psychological factors associated with NAFLD/NASH: a systematic review. European review for medical and pharmacological sciences. 2016; 20(24):5081–97. PMID: 28051283

70. Tomeno W, Kawashima K, Yoneda M, Saito S, Ogawa Y, Honda Y, et al. Non-alcoholic fatty liver disease comorbid with major depressive disorder: The pathological features and poor therapeutic efficacy. Journal of gastroenterology and hepatology. 2015; 30(6):1009–14. https://doi.org/10.1111/jgh.12897 PMID: 25619308

71. Youssef NA, Abdelmalek MF, Binks M, Guy CD, Omenetti A, Smith AD, et al. Associations of depression, anxiety and antidepressants with histological severity of nonalcoholic fatty liver disease. Liver foundation.
61. Sanders RH, Han A, Baker JS, Cobley S. Childhood obesity and its physical and psychological co-morbidities: a systematic review of Australian children and adolescents. European journal of pediatrics. 2015;174(6):715–46. https://doi.org/10.1007/s00431-015-2551-3 PMID: 25922141

62. Raizada MK, Shemer J, Judkins JH, Clarke DW, Masters BA, LeRoith D. Insulin receptors in the brain: structural and physiological characterization. Neurochemical research. 1988;13(4):297–303. PMID: 3292965