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PNPLA3 Gene Polymorphism in Japanese Obese Children

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Background: The genetic background of children with metabolic dysfunction-associated steatotic liver disease (MASLD) in Japan remains unclear. This study aimed to assess the impact of the *PNPLA3* gene polymorphism in Japanese children.

Methods: Thirty-six overweight Japanese children were enrolled. Fatty liver was assessed using abdominal ultrasonography (US) and FibroScan. The *PNPLA3* (rs738409) single-nucleotide polymorphism (SNP) was analyzed to determine allele distribution and to evaluate its association with clinical parameters.

Results: The median age was 12.5 years, and 25 participants were male. The median values for body weight, body mass index (BMI), BMI percentile, and US score were 63.9 kg, 26.1, 96.6, and 6, respectively. The median values for the controlled attenuation parameter (CAP) and liver stiffness measurement (LSM), as assessed by FibroScan, were 292 dB/m and 4.6 kPa, respectively. Notably, 24 carriers of the G allele in the *PNPLA3* (rs738409) polymorphism were identified. However, no significant associations were observed between the presence of the G allele and any clinical parameters.

Conclusions: No clear associations were found between the *PNPLA3* gene polymorphism and clinical features in overweight Japanese children. Further large-scale studies are needed to better understand the genetic factors contributing to MASLD in this population.

Key words: children, metabolic dysfunction-associated steatotic liver disease, *PNPLA3*, single-nucleotide polymorphism

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD), a common chronic liver disorder, affects approximately 30% of adults in Japan and its prevalence has been increasing in recent years[1)2)]. However, data regarding MASLD in children are scarce[3)]. MASLD affects approximately 4–5% of children in Japan; however, the prevalence of pediatric MASLD is likely to increase with the increasing rate of obesity[4)5)].

Lifestyle-related disorders such as obesity, dyslipidemia, and diabetes mellitus have been linked to the etiology of MASLD. However, genetic factors also contribute to its development. A single nucleotide polymorphism (SNP) in patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) (rs738409: C>G, I148M) indicated a higher predisposition to MASLD in a genome-wide association study (GWAS) on patients with MASLD[6)]. GWAS also revealed an association between *PNPLA3* and MASLD progression in Japanese adults[7)8)]. In addition to *PNPLA3*, other genes such as hydroxysteroid 17-beta dehydrogenase 13 (*HSD17B13*) and transmembrane 6 superfamily member 2 (*TM6SF2*)

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also increase susceptibility to MASLD[9)10)]. An association between MASLD and gene polymorphisms, including *PNPLA3*, has also been observed in children[11)-14)]. However, studies exploring the genetic background of MASLD in Japanese children remains lacking. Therefore, this study aimed to investigate the impact of the *PNPLA3* gene polymorphism in Japanese children.

MATERIALS AND METHODS

1. Study participants

Thirty-six overweight Japanese children aged 8–15 years who were referred to the Pediatric Department of Aichi Medical University between November 2020 and January 2024 for evaluation of obesity enrolled in this study. Only 36 cases could be collected during the study period, and data from non-obese (healthy) children were difficult to obtain; therefore, an exploratory study was conducted on these 36 children. A body mass index (BMI) percentile value of 85 or higher was defined as overweight[15)]. BMI percentiles were calculated using the BMI calculation file on the website of the Japanese Society for Pediatric Endocrinology[16)]. Patients with acute or chronic liver diseases (such as viral hepatitis or autoimmune hepatitis), congenital metabolic disorders (such as fatty acid metabolism disorders), endocrine disorders (such as thyroid diseases), or a history of receiving hepatotoxic drug therapy were excluded.

2. Abdominal ultrasonography (US)

Abdominal US examinations were performed by an experienced operator using a Toshiba Aplio 500 Ultrasound System (Toshiba Medical Systems Corporation, Otawara, Japan) equipped with a convex probe (5 MHz). The sum of the following parameters was used to calculate the fatty liver infiltration score: (1) contrast between the liver and kidney parenchyma: 0, 1, and 2 points for unclear, elevated, and markedly elevated contrast, respectively; (2) attenuation of echogenicity in the deep region of the liver: 0, 1, and 2 points for no, moderate, and marked attenuation, respectively; and (3) blurring of liver blood vessel structures: 0, 1, and 2 points for no, moderate, and marked blurring, respectively[17)].

3. FibroScan parameters

Measurements were performed using a FibroScan compact 530® (Echosens, Paris, France). FibroScan examinations were performed using a 3.5 MHz standard M probe (diameter, 7 mm) on the same day as the abdominal US examination. A skilled operator performed the assessment to reduce inter-operator variability. The controlled attenuation parameter (CAP) values (dB/m) were measured as an index of liver fat content. The liver stiffness measurement (LSM) values (kPa) were used to assess liver stiffness. Ten measurements were performed and the median values were analyzed. The FibroScan test was considered successful if 10 valid measurements had a success rate of $\geq$60% and an interquartile range (IQR) of $\leq$30% of the median LSM, as described previously[18)-20)].

4. Demographic and laboratory data

The demographic data assessed during the abdominal US examination included age, sex, height, body weight (BW), BMI, BMI percentile, waist circumference (WC), and percent body fat (PBF). PBF was measured using a direct segmental multifrequency analyzer (InBody H20N, InBody Co., Ltd., Seoul, South Korea).

The laboratory data assessed included the platelet count (PLT); serum levels of total bilirubin (T-BIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cho-

lesterol (LDL-C), fasting blood glucose (FBG), hemoglobin A1c (HbA1c), Mac-2 binding protein glycosylation isomer (M2BPGi), and type IV collagen 7S (COL4-7S).

5. SNP analysis

A Blood Genomic DNA Extraction Mini Kit (FAVORGEN, Pingtung, Taiwan) was used to extract DNA from the peripheral blood of the participants. A Step One Plus real-time PCR system (Applied Biosystems, Foster City, CA, USA) with TaqMan Assays was used to analyze the *PNPLA3* (rs738409) SNP in accordance with the manufacturer's instructions.

6. Statistical analysis

The allele distribution of the *PNPLA3* (rs738409) polymorphism was analyzed in the participants, and its association with clinical parameters was investigated. Categorical and continuous variables were analyzed using Fisher's exact probability test and the Mann-Whitney U test, respectively. Statistical significance was set at a two-sided p-value of ≤ 0.05. All statistical analyses were performed using EZR software, version 1.61(available at http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html)[21].

7. Ethical approval and informed consent statements

The study protocol was approved by the Ethics Committee of Aichi Medical University Hospital (Approval No. 2021-010). This study adhered to the tenets of the Declaration of Helsinki. Written Informed consent was obtained from all participants and/or their caregivers.

RESULTS

Table 1 presents the clinical characteristics of the 36 overweight Japanese children enrolled in this study. The median age was 12.5 years (interquartile range [IQR], 10.6–14.5), and 25 participants were male. The median values for BW, BMI, BMI percentile, WC, and PBF were 63.9 kg (IQR, 56.9–73.6), 26.1 (IQR, 24.3–29.0), 96.6 (IQR, 91.3–98.5), 89.1 cm (IQR, 82.8–95.7), and 37.4% (IQR, 23.8–49.9), respectively. The median US score was 6 (IQR, 4–6). The median values for the CAP and LSM, as assessed by FibroScan, were 292 dB/m (IQR, 246–309) and 4.6 kPa (IQR, 3.8–5.8), respectively.

Table 2 shows the genotype distribution of the *PNPLA3* (rs738409) polymorphism. The genotype frequencies of *PNPLA3* (rs738409) were 33.3% for C/C, 44.5% for C/G, and 22.2% for G/G, respectively. Table 3 presents the clinical characteristics according to *PNPLA3* (rs738409) genotypes. Carriers of the G allele showed no

Table 1. Clinical characteristics of the participants

Number of cases	36
Age (years)	12.5 (10.6–14.5)
Sex (M : F)	25 : 11
Height (cm)	155.2 (146.8–163.5)
BW (kg)	63.9 (56.9–73.6)
BMI	26.1 (24.3–29.0)
BMI percentile	96.6 (91.3–98.5)
WC (cm)	89.1 (82.8–95.7)
PBF (%)	37.4 (23.8–49.9)
US score	6 (4–6)
CAP (dB/m)	292 (246–309)
LSM (kPa)	4.6 (3.8–5.8)

Data are presented as median (interquartile range) for continuous variables.
BW, body weight; BMI, body mass index; WC, waist circumference; PBF, percent body fat; US, ultrasonography; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

Table 2. Genotype distribution of the *PNPLA3* rs738409 polymorphism

Genotype	Number of cases (%)
C/C	12 (33.3%)
C/G	16 (44.5%)
G/G	8 (22.2%)

PNPLA3, patatin-like phospholipase domain-containing protein 3.

Table 3. Clinical characteristics according to *PNPLA3* rs738409 genotypes

	C/C (n=12)	C/G and G/G (n=24)	*p* value
Age (years)	10.7 (10.4–14.0)	13.2 (11.2–14.8)	0.056
Sex (M : F)	8 : 4	17 : 7	>0.99
Height (cm)	155.5 (145.2–164.0)	155.2 (147.8–163.5)	0.775
BW (kg)	65.4 (56.5–74.2)	63.1 (57.2–72.3)	0.84
BMI	28.5 (25.8–30.2)	25.5 (23.6–28.4)	0.144
BMI percentaile	98.1 (96.1–98.7)	95.6 (89.4–97.9)	0.058
WC (cm)	88.3 (83.6–97.8)	89.1 (82.8–93.8)	0.916
PBF (%)	37.3 (33.6–45.7)	37.5 (35.9–45.0)	0.687
US score	6 (4–6)	6 (3–6)	0.604
CAP (dB/m)	297 (246–330)	292 (243–297)	0.248
LSM (kPa)	4.6 (3.8–5.9)	4.8 (3.8–5.6)	0.972
PLT ($10^3/\mu L$)	351 (310–370)	301 (267–360)	0.339
T-BIL (mg/dL)	0.5 (0.4–0.6)	0.6 (0.5–0.7)	0.214
AST (IU/L)	29 (23–55)	31 (22–69)	0.84
ALT (IU/L)	44 (17–109)	38 (17–143)	0.867
γ-GTP (IU/L)	26 (21–46)	23 (14–44)	0.487
TC (mg/dL)	162 (152–170)	165 (151–184)	0.705
LDL-C (mg/dL)	123 (108–130)	125 (102–149)	0.685
TG (mg/dL)	123 (75–182)	116 (63–158)	0.427
FBS (mg/dL)	96 (89–108)	97 (91–106)	0.638
HbA1c (%)	5.6 (5.5–5.7)	5.5 (5.5–5.7)	0.931
M2BPGi	0.66 (0.52–0.82)	0.69 (0.47–0.81)	0.693
COL4-7S (ng/mL)	4.3 (4.2–5.6)	3.9 (3.6–5.3)	0.204

Data are presented as median (interquartile range) for continuous variables.
PNPLA3, patatin-like phospholipase domain-containing protein 3; BW, body weight; BMI, body mass index; WC, waist circumference; PBF, percent body fat; US, ultrasonography; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; PLT, platelet; T-BIL, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; M2BPGi, Mac-2 binding protein glycosylation isomer; COL4-7S, type IV collagen 7S.

significant associations with clinical parameters, including FibroScan measurements and liver fibrosis markers.

DISCUSSION

In this study, we investigated the *PNPLA3* gene polymorphism in overweight Japanese children. To the best of our knowledge, this is the first study to examine this genetic polymorphism in this population. However, the minor G allele of the *PNPLA3* (rs738409) polymorphism was not significantly associated with any clinical parameters.

PNPLA3-encoded adiponutrin, which is localized in lipid droplet membranes, promotes lipase activity and participates in lipid metabolism[22]. The minor allele (G) of a SNP in *PNPLA3* (rs738409: C>G, I148M) inhibits lipase activity and promotes hepatic fat accumulation[23]. The G allele of *PNPLA3* has been implicated in the development of MASLD, metabolic dysfunction-associated steatohepatitis (MASH), fibrosis,

and hepatocarcinogenesis[24)-26)]. *PNPLA3* polymorphism is also associated with the development and progression of MASLD in children. The G allele of *PNPLA3* was associated with elevated ALT and AST levels in a study of 475 children and adolescents with obesity conducted by Romeo et al[11)]. In another study of 520 children with obesity, Lin et al[12)]. reported that the G allele was linked to a higher risk of developing MASLD. In our study, the frequency of *PNPLA3* (rs738409) G allele was 66.7%, which was higher than that reported in the general Japanese population (approximately 42%)[27)]. A study of 520 obese children in Taiwan reported a G allele frequency of 62.7%, which was comparable to our findings[12)]. These results suggest that the *PNPLA3* (rs738409) G allele may influence the development of obesity in childhood. In contrast, no clear associations were observed between the *PNPLA3* (rs738409) G allele and AST/ALT levels, US score, or CAP/LSM values in the present study. Although some studies have reported that G allele carriers tend to have higher levels of liver fibrosis markers such as M2BPGi and COLA7-7S, no significant associations were observed in this study[28)29)]. Given the small sample size, further data are needed to clarify the relationship between the *PNPLA3* genotype and MASLD in Japanese children.

This study had certain limitations. First, the sample size was insufficient to draw broad conclusions. Sophisticated multicenter studies with larger sample sizes must be conducted in the future to draw definitive conclusions. Second, a selection bias may have been introduced during patient enrollment. A population-based survey study design would minimize this bias. Third, histopathological liver data pertaining to the liver could not be obtained. Liver biopsy is an invasive procedure; consequently, obtaining informed consent from pediatric patients and/or their caregivers is difficult. Quantitative evaluation of the liver fat content (CAP) and stiffness (LSM) was performed using FibroScan as surrogate markers. However, reference and cutoff values for CAP and LSM in children have not yet been fully established and are therefore not sufficient[30)]. Fourth, other genetic polymorphisms, such as *HSD17B13* and *TM6SF2*, were not assessed in this study, and thus their potential influence cannot be excluded. Therefore, future studies should include analyses of these and other relevant genetic variants.

In summary, the impact of *PNPLA3* gene polymorphism in overweight Japanese children remains unclear. Further large-scale studies, including liver histological analysis, are warranted to clarify its clinical significance.

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Declaration of conflicting interest

The authors declare no conflicts of interest associated with this manuscript.

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