INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most unabating cause of chronic liver disease in children and adolescents. NAFLD is a broad spectrum of disease manifested by hepatic fat accumulation. It varies from simple steatosis to non-alcoholic steatohepatitis and may even develop cirrhosis. The prevalence of paediatric NAFLD has increased up to 10%, and this rises to 40-70% among obese individuals. Various epidemiological, familial and twin studies have concluded that genetic factors may also play a role in determining the vulnerability to non-alcoholic steatohepatitis.

Single nucleotide polymorphisms in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) genes involved in hepatic inflammation, oxidative stress, insulin signalling, as well as fibrogenesis have been associated with the histological severity of liver damage in NAFLD. A genome-wide association study revealed that the rs738409 polymorphism in PNPLA3 is strongly associated with hepatic fat content and fibrosis. PNPLA3 encodes a 481 amino acid protein that is notably expressed in the liver. The rs7384089 single nucleotide polymorphism in the PNPLA3 gene is a missense variation, in particular isoleucine to methionine substitution at amino acid 148 (I148). It results in the abolishment of triglyceride lipase activity leading to increased intracellular triglyceride content.

The risk allele (G allele) frequencies of the rs738409 single nucleotide polymorphisms are concordant with the prevalence of NAFLD in various ethnic groups such as Hispanics, Europeans, African Americans and Asians.
There is a scarcity of data on PNPLA3 polymorphism in the Bangladeshi paediatric population. Therefore, this study was done to examine the relationship of PNPLA3 polymorphism with NAFLD in overweight children.

METHODS

This case-control study was performed in overweight Bangladeshi children from June 2021 to December 2022. We recruited consecutive children who attended the outpatient and inpatient departments of Paediatric Gastroenterology and Paediatric Endocrinology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. After obtaining informed consent from responsible guardians and assent from patients, we started recruitment. Inclusion criteria were all overweight children (aged 6-17 years) where body mass index ≥85th percentile to <95th percentile was defined as overweight for children and teenagers of the same age and sex. Exclusion criteria were (i) acute hepatitis in the previous six months; (ii) unusual thyroid profile; (iii) known and overt case of any other liver diseases i.e. chronic viral hepatitis (hepatitis B and hepatitis C), autoimmune liver diseases, Wilson's disease, hereditary hemochromatosis, cirrhosis of the liver; (iv) severe malnutrition; (v) syndromic obesity; (vi) history of taking drugs that may cause fatty liver like steroids, amiodarone, methotrexate, tamoxifen and antitubercular therapy.

Based on the above criteria, 55 children were included. Among them, 4 children refused to give consent. Finally, 51 children were divided into cases and controls according to the presence of fatty liver by ultrasonography. Thirty-one overweight children with NAFLD were included in the case group and 20 who had no NAFLD were in the control group. All the children were tested for secondary causes that cause fatty liver. Acute viral hepatitis was ruled out using the appropriate tests (anti-HAV IgM, anti-HEV IgE, anti-HBcIgM).

Biochemical and anthropometric measurements

Previous family and medical history were assessed in all children during physical examination by the physician. The lipid profiles were assessed using morning blood samples obtained after about about 12-hour of fasting. Fasting and 2 hours after breakfast blood glucose, alanine aminotransferase measurements were done. For the PNPLA3 genotyping assay, 3 ml of blood was taken. All samples were stored at -20°C until use. Real-time polymerase chain reaction was performed on the Stratagene Mx3005P instrument (held at 95°C for 10 min, followed by 40 cycles of 95°C for 15 second, and 60°C for 45 second) with an single nucleotide polymorphisms genotyping assay for rs738409 by using predesigned Taqman single nucleotide polymorphisms genotyping kit. Human PNPLA3 rs738409 is bi-allelic with three possible genotypes as follows: C/C, homozygous wild-type; C/G, heterozygous; and G/G, homozygous variant.

Statistical analysis

Data were analyzed by Statistical Package for the Social Sciences software for Windows version 22.0. Data were expressed as number (percent) and mean (standard deviations) for categorical and quantitative variables, respectively. NAFLD was graded as Grade 1: when there is a slight and diffuse increase of liver echogenicity with normal visualization of the diaphragm and of the portal vein wall; Grade 2: moderate increase of liver echogenicity with slightly impaired appearance of portal vein wall and the diaphragm; and Grade 3: marked increase of liver echogenicity with poor or no visualization of portal vein wall, diaphragm and right lobe of liver. Analysis was done by independent t test (Mann–Whitney U test for non-normal data) for quantitative variables and Chi-square test for categorical variables. Univariate and multivariate logistic regression analysis were done to examine the association between NAFLD and PNPLA3 genotypes. Age, sex, body mass index, alanine aminotransferase, and triglycerides which exhibited statistically significant associations were included in the multivariate analysis.
differences between the case and control groups were adjusted in the multivariate model and the G/G genotype was considered as the reference category. P<0.05 was considered as statistically significant.

RESULTS

The mean (standard deviation) age of the participants was 10.8 (2.9) years and significantly different between cases and controls (P=0.04). More than two-thirds (68.6%) were boys. The mean (standard deviation) body mass index was 26.7 (4.1) kg/m² and significantly different between cases and controls (P=0.01).

Among the participants, 62% exhibited acanthosis. The mean (standard deviation) haemoglobin, fasting blood sugar, alanine aminotransferase, triglycerides and total cholesterol level were 12.5 (1.4) gm/dl, 5.1 (0.7) mg/dl, 178.8 (95.4) mg/dl and 177.3 (53.7) mg/dl, respectively. However, only alanine aminotransferase and triglycerides were found to be significantly different between cases and controls (P<0.001).

The mean (standard deviation) PNPLA3 genotype was considered as the reference category. The odds ratios for cases in relation to PNPLA3 genotypes were 5.0 (95% CI 0.06, 453.0) for C/C and 1.8 (95% CI 0.04, 91.0) for C/G in the multivariate logistic regression model, adjusted for age, sex, body mass index, alanine aminotransferase, and triglycerides but not statistically significant. Notably, no significant association was observed between the genotypes and the presence of Grades 1 and 2 of NAFLD (FIGURE 1).

DISCUSSION

This study investigated the association between the PNPLA3 gene and NAFLD in Bangladeshi overweight children. The study identified a noteworthy association between specific PNPLA3 gene polymorphisms, particularly the C/G and C/C genotypes, and an increased risk of NAFLD among Bangladeshi overweight children. Although the odds ratios did not reach statistical significance, this trend suggests a potential genetic predisposition to NAFLD within this population. Importantly, no significant association was found between PNPLA3 genotypes and the severity of NAFLD, as assessed by Grades 1 and 2 of fatty liver disease. However, body mass index, alanine aminotransferase and triglycerides were found to be higher in the NAFLD group.

Several studies, mostly in adults, have detected a strong association between PNPLA3 polymorphism with hepatic steatosis as well as steatohepatitis. Results vary among these studies, and further research is needed to understand the role of PNPLA3 in NAFLD in children. Overall, this study highlights the importance of considering genetic factors in the development of pediatric NAFLD and underscores the need for additional research to elucidate the genetic basis of this condition in children.
from study to study in paediatric populations with overweight or obesity. Our finding is similar to a study done in India where 69.9% of obese and overweight children were found to have NAFLD. Huang et al. showed that higher body mass index and alanine aminotransferase were independent predictors of NAFLD in children as we also observed.

Previous studies from several geographical regions also reported serum total cholesterol and triglycerides levels are significantly higher in paediatric NAFLD. Serum alanine aminotransferase had been used as a screening test to early pick up paediatric NAFLD cases.

In the present study, we did not find any association between homozygosity for PNPLA3 polymorphism and NAFLD. Sood et al. found similar results. This may be because NAFLD is a multi-dimensional disease. Various risk factors like dietary habits, physical activity, and metabolic or genetic polymorphism of the PNPLA3 gene may play a role in the development of paediatric NAFLD. A series of studies showed an association between rs738409 single nucleotide polymorphism in the PNPLA3 gene with hepatic steatosis and its severity in adult as well as the paediatric age group. This has been further supported by a recent meta-analysis.

Our study has a limitation in diagnosing NAFLD by ultrasonography, which has been accepted as the first-line screening test. An invasive procedure of liver biopsy is necessary to confirm the diagnosis of NAFLD, but we could not do it. We do not have diet and physical activity data either. These reasons exhibit a lack of generalizability. However, this study contributes to the limited pool of research on the association between the PNPLA3 gene and pediatric NAFLD in the Bangladeshi population, adding valuable data to the global knowledge base.

**Conclusion**

In his study, we did not uncover any significant association between the PNPLA3 rs738409 single nucleotide polymorphism and the presence of NAFLD in children. However, our findings suggest that elevated levels of alanine aminotransferase and triglycerides may serve as an early screening indicators for NAFLD in overweight children.

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**Author Contributions**

Conception and design: RA, MR, KLN, SM. Acquisition, analysis, and interpretation of data: RA, KF, SM, LA. Manuscript drafting and revising it critically: RA, KF, MR, KLN, SM, LA, ANIS. Approval of the final version of the manuscript: RA, KF, MR, KLN, SM, LA, ANIS. Guarantor accuracy and integrity of the work: RA, KF, MR, KLN, SM, LA, ANIS.

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**Conflict of Interest**

The authors have no conflict of interest to declare.

**Ethical Approval**

Prior to the commencement of this study, ethical clearance was taken from the Institutional Review Board of the BSMMU. Approval was given on 18 January 2020 (Memo No. BSMMU/2020/1102).

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