

Adenosine deaminase and interleukin-1 receptor antagonist genetic polymorphisms among obese children with versus without metabolic dysfunction-associated fatty liver disease

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Background: Metabolic disorder-associated fatty liver disease (MAFLD) in children is an emerging global health concern, particularly in terms of obesity and metabolic disturbances. Inflammation plays a crucial role in the pathogenesis of MAFLD, with adenosine deaminase (ADA) and interleukin-1 receptor antagonist (IL-1Ra) being potential contributors.

Purpose: This study aimed to assess the association between ADA G22A and IL-1RN single nucleotide polymorphisms (SNPs) and MAFLD among a cohort of Egyptian children. It also aimed to evaluate the validity of very low-density lipoprotein (VLDL)/high-density lipoprotein cholesterol (HDL-C) and triglyceride-to-HDL-C ratios for predicting MAFLD in obese children.

Methods: One hundred obese children and 50 healthy controls were included. The obese group was further categorized into those with versus without MAFLD. IL-1Ra and ADA G22A SNPs were evaluated using conventional polymerase chain reaction (PCR) and restriction fragment length polymorphism-PCR, respectively. VLDL/HDL and triglyceride-to-HDL ratios were calculated from the lipid profiles of the included participants.

Results: The obese children had significantly higher weight, weight z score, body mass index (BMI), BMI z score, and waist circumference than the healthy controls. These parameters were considerably higher in children with versus without MAFLD $P < 0.05$ all. The GG genotype and G allele of ADA G22A were significantly more frequent in the obese children versus controls ($P < 0.05$ for both); however, no significant difference was observed between obese children with versus without MAFLD. Regarding IL-1RN polymorphisms, the *2/*2 genotype was more common in the controls and obese children without MAFLD, whereas the *1/*2 genotype was prevalent in the obese children

with MAFLD ($P < 0.05$ all). A VLDL/HDL-C cutoff ratio of > 0.6308 showed 80% sensitivity, 58% specificity, a 65.6% positive predictive value (PPV), a 74.4% negative predictive value, and 69% accuracy at differentiating among MAFLD cases. The triglyceride-to-HDL-C ratio cutoff of > 3.0685 demonstrated high specificity (88%) and a high PPV (84.2%) but moderate sensitivity (64%) and overall accuracy (76%).

Conclusion: The current study's findings support the possible genetic role of ADA G22A in childhood obesity, with a significant role for the IL-1RN SNP in the development of MAFLD in obese children. The triglyceride-to-HDL-C ratio was more useful than the VLDL/HDL-C ratio for predicting pediatric MAFLD.

Key words: Adenosine deaminase, Genetic polymorphism, Interleukin 1 receptor antagonist protein, VLDL cholesterol, HDL cholesterol, Triglycerides, Metabolic dysfunction-associated fatty liver disease

Key message

Question: Is there an association between adenosine deaminase (ADA) G22A and interleukin-1 receptor antagonist (IL-1RN) genetic polymorphisms and pediatric metabolic dysfunction-associated fatty liver disease (MAFLD)?

Finding: The GG genotype and G allele of ADA G22A were significantly associated with obesity but not pediatric MAFLD, while the *1/*2 genotype of the IL-1RN gene was significantly associated with obesity and pediatric MAFLD.

Meaning: The IL-1RN gene may contribute to pediatric MAFLD.

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Introduction

Metabolic-associated fatty liver disease (MAFLD), previously known as nonalcoholic fatty liver disease (NAFLD), has become the most prevalent cause of chronic liver disease among children and adolescents.¹⁾ The global prevalence of MAFLD in this demographic has risen alarmingly, paralleling the increase in pediatric obesity rates. Notably, obesity is a major contributor to MAFLD, with prevalence rates of 34% among overweight and obese children and adolescents aged 1-19 years.²⁾

Beyond environmental factors, genetic predispositions play a significant role in MAFLD susceptibility. Interleukin-1 receptors (IL-1Rs) are integral to the inflammatory response, mediating the effects of interleukin-1 cytokines. Research indicates that IL-1 β , a key cytokine interacting with IL-1Rs, plays a significant role in obesity-related liver conditions. In studies involving IL-1 β knockout mice subjected to high-fat diets, these mice exhibited increased adipose tissue expansion and reduced liver weight compared to wild-type controls. This suggests that IL-1 β contributes to adipose tissue inflammation and limits its expandability, leading to ectopic fat deposition in the liver, thereby promoting hepatic steatosis.³⁾

Interleukin-1 receptor antagonist (IL-1Ra), encoded by the *IL1RN* gene, plays a pivotal role in modulating inflammatory responses by inhibiting the activities of interleukin-1 cytokines. IL-1 family cytokines, including IL-1Ra, are integral to liver inflammation. Dysregulation of IL-1 signaling contributes to the progression of NAFLD, now commonly referred to as MAFLD. Elevated IL-1Ra levels have been observed in individuals with MAFLD, implicating its role in disease pathogenesis.⁴⁾ Therapeutic Potential targeting IL-1 pathways, particularly using IL-1 inhibitors, has been proposed as a therapeutic strategy for MAFLD. Modulating IL-1Ra levels may ameliorate liver inflammation and improve metabolic outcomes in affected individuals.⁵⁾

Adenosine deaminase (ADA) is crucial for purine metabolism, converting adenosine into inosine. Its activity influences extracellular adenosine levels, which interact with adenosine receptors affecting various metabolic pathways. Alterations in adenosine signaling have been implicated in obesity and liver diseases. For instance, activation of A1 adenosine receptors stimulates adipogenesis and lipid accumulation, promoting weight gain, whereas A2B receptor activation reduces adipogenesis and insulin resistance.⁶⁾ Furthermore, studies have shown that adenosine generated by ethanol metabolism contributes to hepatic steatosis via both A1 and A2B receptors, suggesting that targeting adenosine receptors may be effective in preventing alcohol-induced fatty liver.⁷⁾

The aim of the current study was to assess the relationship between the development of metabolically related fatty liver and the ADA G22A and IL-1NR gene polymorphisms in obese Egyptian children and adolescents.

Methods

1. Study design and participants

A case-control study in line with the Declaration of Helsinki's principles was performed with 100 obese Egyptian children and adolescents, and 50 healthy control participants. The obese group was further categorized into children with and without MAFLD. Approval from Qena University's ethics committee with an ethical approval code (SVUMEDPED025425127) was obtained. Cases were selected from the outpatient pediatric endocrinology clinics of Qena University Hospitals, South Valley University, Upper Egypt. A signed approval was obtained from participants above 16 years, while caregivers provided consent for children under 16. Sample size was adjusted to achieve 80% power and 5% confidence of significance (type I error).

Inclusion criteria: Obese children and adolescent aged from 6 to 18 years with or without MAFLD. Obesity was diagnosed based on body mass index (BMI) z score > +2 standard deviations.^{8,9)} The existence of hepatic steatosis and leastwise one of the subsequent criteria was used to classify children as having MAFLD: excess adiposity (overweight/obese or abdominal obesity), prediabetes or type 2 diabetes mellitus, and metabolic abnormalities.¹⁰⁾

Exclusion criteria: Patients were excluded if associated with autoimmune diseases, positive hepatitis B/C infections, concurrent chronic illnesses, or patients receiving drug-induced liver injury, or patients whom caregivers refused to participate in the study.

2. Clinical evaluation of the participants

All participants were subjected to complete history-taking and complete physical examinations.

Depending upon abdominal ultrasonography which was performed with 2 specialized radiologists; at least 2 of the following findings are necessary for the diagnosis of hepatic steatosis: vascular blurring, deep attenuation of the ultrasound signal, and a liver with diffusely elevated echogenicity (or "bright") appearing more echogenic than the kidney or spleen.¹¹⁾

Metabolic abnormalities in children were identified as the existence of leastwise 2 of the subsequent demands: (1) plasma triglyceride (TG) levels more than 150 mg/dL, (2) plasma high-density lipoprotein cholesterol (HDL-C) less than 40 mg/dL, (3) TG/HDL-C ratio more than 2.25, and (4)

hypertension with systolic blood pressure more than 130 mmHg or diastolic blood pressure more than 85 mmHg. The MAFLD adult standards were applied to youngsters older than 15.¹²⁾

3. Laboratory investigations

1) Routine laboratory investigations

Five milliliters of venous blood were obtained from all participants and separated into 2 samples; the first (3 mL) was collected in a plain vacutainer tube, centrifuged, and the resultant sera were utilized for routine biochemical analyses in the form of liver enzymes (alanine transaminase [ALT] and aspartate transaminase [AST], lipid profile (total cholesterol, TG, very low-density lipoprotein [VLDL], HDL, and low-density lipoprotein [LDL]; VLDL/ HDL-C and TG/HDL-C ratios were calculated), glycemic profile (random blood glucose, fasting blood glucose, 2-hour postprandial glucose levels, glycated hemoglobin [HbA1c], homeostatic model assessment for insulin resistance [HOMA-IR]), and serum cortisol levels. While the second (2 mL) was collected in a tube containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) and employed for complete blood count.

2) Genetic analysis

(1) DNA Extraction and Genotyping of ADA G22A Polymorphism

Venous blood samples (2 mL) were collected in EDTA tubes and stored at -80°C until genetic analysis. DNA was extracted using the G-spin Total DNA Extraction Kit (iNtRON Biotechnology, Korea) and stored at -80°C. Genotyping of the ADA G22A polymorphism was performed using restriction fragment length polymorphism-polymerase chain reaction (PCR). The PCR reaction (25 µL) contained 12.5-µL PCR master mix (catalog no. 25028, iNtRON Biotechnology), 1 µL of forward primer (5'-GCCCCGCCCCGTTAAGAAGAGC-3'), 1 µL of reverse primer (5'-GGTCAAGTCAGGGGCAGAAGCAGA-3'),^{13,14)} 8.5 µL nuclease-free water, and 2 µL of extracted DNA. Thermal cycling conditions included an initial denaturation at 94°C for 15 minutes, followed by 36 cycles of denaturation (94°C, 40 seconds), annealing (66°C, 80 seconds), and extension (72°C, 80 seconds), with a final extension at 72°C for 8 minutes using an Applied Biosystems thermal cycler (Perkin-Elmer 9600, USA).

PCR products were digested with TaqI enzyme (Cat. No. ER0671, Thermo Fisher Scientific, USA) in a reaction containing 1-µL enzyme, 2-µL buffer, 7-µL nuclease-free water, and 10-µL PCR product, incubated at 65°C for 1 hour. The digested fragments were resolved on a 2% agarose gel stained with ethidium bromide and visualized under

a UV transilluminator. A 50-bp DNA ladder (Catalog No. 10416014, Thermo Fisher Scientific) was used for band size determination. The ADA G22A genotype was identified based on banding patterns: ADA1/ADA1 (GG) showed 2 bands (245 and 152 bp), while ADA1/ADA2 (GA) displayed 3 bands (397, 245, and 152 bp) (Fig. 1).

(2) Genotyping of interleukin-1 receptor antagonist (*IL-1RN*) gene polymorphism

Genotyping of *IL-1RN* gene polymorphism was performed using conventional PCR without restriction enzyme digestion. The primers used (GeneBank accession no. X64532) were: sense (5'-CTCAGCCAACACTCCTAT-3') and antisense (5'-TCCTGGTCTGCAGGTAA-3'), as described by Swellam et al.¹⁵⁾ The PCR reaction (25 µL) included 12.5-µL PCR master mix (catalog no. 25028, iNtRON Biotechnology), 1 µL each of forward and reverse primers, 8.5-µL nuclease-free water, and 2 µL of extracted DNA. Thermal cycling conditions consisted of an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation (94°C, 1 minute), annealing (°C, 1 minute), and extension (72°C 1 minute), with a final extension at 72°C for 10 minutes. Reactions were conducted using a Biometra thermal cycler (Germany, serial number 2603204). PCR products were resolved on a 2% agarose gel stained with ethidium bromide, along with a 50 bp DNA ladder (Catalog No. 10416014, Thermo Fisher Scientific). Electrophoresis was performed at 120 V for 15–20 minutes, and bands were visualized under UV light using a gel documentation system. The *IL-1RN* alleles were identified based on product sizes: *IL-1RN0* (150 bp), *IL-1RN1* (410 bp), *IL-1RN2* (240 bp), *IL-1RN3* (325 bp), *IL-1RN4* (500 bp), and *IL-1RN5* (595 bp).

4. Statistical analysis

The study analyzed data using IBM SPSS Statistics ver. 27.0 (IBM Co., USA). Normality was evaluated with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous variables were reported as mean±standard deviation (parametric) or median and interquartile range (nonparametric). Group differences were tested using independent-samples *t* test (parametric) and Mann-Whitney *U* test (nonparametric) for 2-group comparisons. Kruskal-Wallis *H* test for comparisons among 3 or more groups (due to nonnormal distribution). Chi-square test for categorical variables. Receiver operating characteristic curve analysis determined cutoff values for VLDL/HDL and TG/HDL ratios, assessing their diagnostic performance. Logistic regression identified independent risk factors for MAFLD in children. The studied SNPs followed Hardy-Weinberg equation. A *P* value <0.05 was considered statistically significant.

Results

This study compared 100 obese children with 50 age- and sex-matched healthy controls (median age: 12.0 years vs. 12.1 years; sex distribution: 38% vs. 40% males, 62% vs. 60% females). Obese children had significantly higher weight (73 kg vs. 54.5 kg, $P<0.001$), weight z score (2.52 vs. 0.41, $P<0.001$), BMI (33.69 kg/m² vs. 20.82 kg/m², $P<0.001$), BMI z score (2.5 vs. 0.5, $P<0.001$), and waist circumference (89 cm vs. 67.5 cm, $P<0.001$). These findings confirm a strong association between obesity and central adiposity. Lower hemoglobin levels in obese children (11.06±1.32 g/dL vs. 11.78±0.97 g/dL, $P<0.001$). Higher insulin resistance

(HOMA-IR: 2.6 vs. 1.01, $P<0.001$), strongly indicating metabolic dysfunction. Elevated post meridiem (PM) cortisol levels ($P<0.001$), suggesting altered diurnal cortisol regulation. AST and ALT are significantly higher in patients ($P=0.032$ and $P<0.001$, respectively) and AST/ALT ratio is significantly lower in obese children (0.91 vs. 1.28, $P<0.001$), indicating altered liver enzyme balance. Obese children had significantly higher total cholesterol, LDL, VLDL/ HDL ratio, and TGs/ HDL ratio ($P<0.001$). Obese children had significantly lower VLDL and HDL (Table 1).

Table 2 compares obese children with MAFLD to those without, highlighting greater obesity severity, metabolic dysfunction, inflammation, and liver injury in the MAFLD

Table 1. Clinical and laboratory data of study participants

Variable	Patients (n=100)	Controls (N=50)	P value
Age (yr)	12.0 (9.0–14.2)	12.1 (10.0–14.6)	0.445
Sex			
Male	38 (38)	20 (40)	0.813
Female	62 (62)	30 (60)	
Weight (kg)	73 (62–85)	54.5 (32.0–60.0)	<0.001
Weight z score	2.52 (2.12–2.97)	0.41 (0.07–0.57)	<0.001
Height (cm)	145.00 (135.38–155.75)	150.5 (135.0–161.0)	0.482
Height z score	0.26 (–0.58 to 1.2)	–0.02 (–0.47 to 0.22)	0.072
BMI (kg/m ²)	33.69 (31.1–36.18)	20.82 (17.39–22.45)	<0.001
BMI z score	2.50 (2.18–2.68)	0.50 (0.26–0.77)	<0.001
Waist circumference (cm)	89.00 (83.00–97.88)	67.50 (58.75–71.25)	<0.001
CBC parameter			
Hgb (g/dL)	11.06±1.32	11.78±0.97	0.001
WBCs (×10 ⁹ /L)	6.84 (5.43–9.30)	7.20 (5.45–9.43)	0.812
Platelet (×10 ⁹ /L)	252.5 (220.0–335.0)	250.0 (212.0–331.3)	0.697
Glycemic profile			
Random blood glucose (mg/dL)	125 (111–135)	120 (115–130)	0.618
Fasting blood glucose (mg/dL)	95 (80–107)	85 (78.75–94)	0.001
2-Hr postprandial blood glucose (mg/dL)	130 (120–135)	135 (130–135)	0.144
HOMA-IR	2.6 (1.97–2.9)	1.01 (0.77–1.33)	<0.001
HbA1c (%)	5.2 (4.9–5.59)	5.1 (4.9–5.4)	0.210
Serum cortisol AM (μg/dL)	8.4 (6.23–12.15)	8.2 (5.6–11.05)	0.202
Serum cortisol PM (μg/dL)	10.25 (5.3–13.35)	5.9 (4.35–9.2)	<0.001
Liver enzymes			
AST (U/L)	32 (27–35)	27.75 (24–31)	0.032
ALT (U/L)	32 (30–37)	21 (18–22.25)	<0.001
AST/ALT ratio	0.91 (0.81–1.10)	1.28 (1.14–1.56)	<0.001
Lipid profile			
Total cholesterol (mg/dL)	200.5 (82.0–220.0)	98.50 (78.95–115.00)	<0.001
Triglycerides (mg/dL)	109.0 (79.5–118.0)	110.5 (100.0–115.0)	0.231
VLDL (mg/dL)	23 (20–34)	30.59 (28.5–36.63)	<0.001
HDL (mg/dL)	34.5 (30.0–39.4)	52.00 (48.75–57.48)	<0.001
VLDL/HDL-C ratio	0.73 (0.56–0.93)	0.60 (0.51–0.72)	0.006
LDL (mg/dL)	141.23 (26.93–167.00)	24.90 (10.98–44.40)	<0.001
TG/ HDL-C ratio	2.83 (2.25–3.87)	1.93 (1.76–2.13)	<0.001

Values are presented as median (interquartile range) or number (%).

BMI, body mass index; CBC, complete blood count; Hgb, hemoglobin; WBC, white blood cell; HOMA-IR, homeostatic model assessment for insulin resistance; HbA1c, glycated hemoglobin; AM, ante meridiem; PM, post meridiem; AST, aspartate aminotransferase; ALT, alanine aminotransferase; VLDL, very low-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

Boldface indicates a statistically significant difference with $P<0.05$.

group. Higher weight (80 kg vs. 62 kg, $P<0.001$) and BMI (36.15 kg/m² vs. 31.12 kg/m², $P<0.001$) in the MAFLD group. Higher weight z score & BMI z score ($P<0.001$), confirming greater obesity severity. Larger waist circumference (89.5 cm vs. 85 cm, $P<0.001$), indicating stronger central obesity association. Laboratory data showed a higher white blood cell (WBC) count ($P=0.001$), suggesting increased inflammation, and higher platelet count ($P<0.001$), possibly linked to liver dysfunction. Greater insulin resistance (HOMA-IR, $P=0.016$) and higher HbA1C levels ($P=0.025$), indicating metabolic disturbances. Significant higher AST and ALT levels in the MAFLD group. Lipid profile showed higher total cholesterol and LDL ($P<0.001$), reflecting a

more atherogenic profile. Higher VLDL/HDL-C ratio ($P=0.002$) and TG/HDL-C ratio ($P<0.001$), suggesting increased cardiovascular risk. Lower HDL ($P<0.001$), increasing cardiovascular risk.

Table 3 examines the genetic distribution of ADA G22A polymorphisms among patients and controls, as well as between obese children with and without MAFLD. GG genotype is significantly more frequent in patients (38%) than in controls (14%) ($P=0.002$), suggesting a strong association with disease susceptibility. GA genotype is more common in controls (86%) than in patients (62%), implying a potential protective effect of the GA variant. G allele is significantly more frequent in patients (69%)

Table 2. Clinical and laboratory data by study subgroup

Variable	Obese children with MAFLD (n=50)	Obese children without MAFLD (n=50)	P value
Age (yr)	12.0 (10.0–13.9)	10.0 (8.5–14.5)	0.116
Sex			
Male	18 (36)	20 (40)	0.680
Female	32 (64)	30 (60)	
Weight (kg)	80.0 (70.0–98.8)	62.0 (52.0–75.5)	<0.001
Weight z score	2.82 (2.46–3.10)	2.38 (1.82–2.76)	<0.001
Height (cm)	149.5 (138.0–157.0)	140.7 (135.0–153.5.0)	0.191
Height z score	0.03 (-0.58 to 1.29)	0.36 (-1.18 to 1.18)	0.694
BMI (kg/m ²)	36.15 (34.63–41.60)	31.12 (30.00–32.90)	<0.001
BMI z score	2.66 (2.47–2.80)	2.18 (2.13–2.52)	<0.001
Waist circumference (cm)	89.50 (86.00–116.75)	85.00 (80.00–93.13)	<0.001
CBC parameter			
Hgb (g/dL)	11.20±1.32	10.93±1.31	0.307
WBCs (×10 ⁹ /L)	8.50 (5.68–10.43)	6.50 (5.30–8.03)	0.001
Platelet (×10 ⁹ /L)	320.5 (250.0–365.3)	229.0 (206.0–262.5)	<0.001
Glycemic profile			
Random blood glucose (mg/dL)	128.5 (110.0–135.0)	124.0 (112.5–130.8)	0.300
Fasting blood glucose (mg/dL)	95 (85–107)	93.0 (80.0–103.3)	0.222
2-Hr postprandial blood glucose (mg/dL)	130 (127–137)	129.5 (120.0–135.0)	0.085
HOMA-IR	2.8 (2.0–2.9)	2.3 (1.9–2.7)	0.016
HbA1c (%)	5.30 (5.08–5.62)	5.1 (4.8–5.5)	0.025
Serum cortisol AM (μg/dL)	8.20 (5.43–10.55)	8.75 (5.60–11.13)	0.767
Serum cortisol PM (μg/dL)	9.1 (4.9–13.8)	10.3 (5.3–13.0)	0.849
Liver enzymes			
AST (U/L)	32.0 (28.8–43.0)	30.5 (25.8–35.0)	0.010
ALT (U/L)	35.0 (31.6–40.3)	31.0 (30.0–33.3)	<0.001
AST/ALT ratio	0.91 (0.81–1.10)	0.94 (0.81–1.09)	0.664
Lipid profile			
Total cholesterol (mg/dL)	219.5 (202.8–235.0)	158.5 (79.4–200.3)	<0.001
Triglycerides (mg/dL)	111.0 (75.8–121.3)	101.5 (88.3–115.0)	0.388
VLDL (mg/dL)	22.0 (18.9–28.0)	25 (20–35)	0.187
HDL (mg/dL)	30.5 (27.0–32.3)	39.1 (36.5–43.3)	<0.001
VLDL/HDL-C ratio	0.75 (0.67–1.01)	0.61 (0.50–0.78)	0.002
LDL (mg/dL)	167.0 (151.0–183.9)	33.86 (18.85–133.45)	<0.001
TG/ HDL-C ratio	3.66 (2.58–4.19)	2.40 (1.92–2.93)	<0.001

Values are presented as median (interquartile range) or number (%).

BMI, body mass index; CBC, complete blood count; Hgb, hemoglobin; WBC, white blood cell; HOMA-IR, homeostatic model assessment for insulin resistance; HbA1c, glycated hemoglobin; AM, ante meridiem; PM, post meridiem; AST, aspartate aminotransferase; ALT, alanine aminotransferase; VLDL, very low-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

Boldface indicates a statistically significant difference with $P<0.05$.

versus controls (57%) ($P=0.040$), indicating an increased disease risk associated with the G allele. GG genotype (odds ratio [OR], 3.765; 95% confidence interval [CI], 1.538–9.215), meaning patients are ~3.76 times more likely to have this genotype and G allele (OR, 1.679; 95% CI, 1.022–2.759) suggesting a moderate association with disease risk. No significant differences in ADA G22A genotype or allele distributions between the 2 groups with and without MAFLD.

Table 4 presents the genetic profile of IL-1RN polymorphisms among different studied groups, focusing on genotype and allele distributions. Comparison between patients and controls showed that the *1/*1 genotype is more frequent in controls (66%) than in patients (61%). The *1/*2 genotype appears in 25% of patients versus (4%) in controls. The *2/*2 genotype is found in 14% of patients versus 30% in controls. The genotype distribution shows a statistically significant difference ($P<0.001$), suggesting an association between IL-1RN polymorphism and disease presence. Comparison between obese children with and without MAFLD revealed that the *1/*1 genotype is less frequent in obese children with MAFLD (58%) compared to those without MAFLD (64%). The *1/*2 genotype is more common in obese children with MAFLD (38%)

than those without (12%). The *2/*2 genotype is more frequent in obese children without MAFLD (24%) than in those with MAFLD (4%). The genotype distribution is significantly different ($P<0.001$), indicating a potential role of IL-1RN polymorphism in MAFLD development. The allele frequencies (ILRN1 vs. ILRN2) show no significant difference ($P=0.262$), suggesting that specific genotypes, rather than alleles alone, might be influencing MAFLD risk.

Table 5 presents the mean lipid profile parameters for different genotypes in relation to the 2 gene polymorphism. VLDL is significantly higher in GA (26.5 mg/dL) than in GG (21 mg/dL) ($P<0.001$) and HDL is also higher in GA (35.05 mg/dL) than in GG (31.95 mg/dL) ($P=0.049$). No significant differences in other lipid profile. Lipid profile analysis in relation to IL-1RN Genotype showed that HDL is highest in *2/*2 (39.1 mg/dL), followed by *1/*1 (33 mg/dL) and *1/*2 (32.8 mg/dL) ($P=0.004$), suggesting *2/*2 is linked to better HDL levels. LDL is highest in *1/*2 (155 mg/dL), lowest in *2/*2 (31.47 mg/dL) ($P=0.045$), indicating a possible protective effect of *2/*2. No significant differences in total cholesterol, TGs, VLDL, VLDL/HDL ratio, or TGs/HDL ratio.

Fig. 2 showed that VLDL/HDL-C ratio is fair (area under the curve [AUC]=0.68) to discriminate Obese children with

Table 3. Genetic profile of ADA G22A polymorphisms among the study participants

Study group	ADA G22A genotypes		Alleles	
	GG	GA	G	A
Patients (n=100)	38 (38)	62 (62)	138 (69)	62 (31)
Controls (N=50)	7 (14)	43 (86)	57 (57)	43 (43)
P value (χ^2)	0.002 (9.143)		0.040 (4.220)	
OR (95% CI)	3.765 (1.538–9.215)		1.679 (1.022–2.759)	
Obese children with MAFLD (n=50)	18 (36)	32 (64)	68 (68)	32 (32)
Obese children without MAFLD (n=50)	20 (40)	30 (60)	70 (70)	30 (30)
P value (χ^2)	0.680 (0.170)		0.760 (0.094)	
OR (95% CI)	0.844 (0.376–1.894)		0.911 (0.500–1.659)	

Values are presented as number (%) unless otherwise indicated.

ADA, adenosine deaminase; OR, odds ratio; CI, confidence interval; MAFLD, metabolic dysfunction-associated fatty liver disease.

Boldface indicates a statistically significant difference with $P<0.05$.

Table 4. Genetic profile of IL-1RN polymorphisms among study participants

Study group	IL-1RN genotypes			Alleles	
	*1/*1	*1/*2	*2/*2	IL-RN*1	IL-RN*2
Patients (n=100)	61 (61)	25 (25)	14 (14)	147 (73.5)	53 (26.5)
Controls (N=50)	33 (66)	2 (4)	15 (30)	70 (70)	30 (30)
P value (χ^2)	<0.001 (17.336)			0.523 (0.408)	
OR (95% CI)	-			1.189 (0.699–2.021)	
Obese children with MAFLD (n=50)	29 (58)	19 (38)	2 (4)	77 (77)	23 (23)
Obese children without MAFLD (n=50)	32 (64)	6 (12)	12 (24)	70 (70)	30 (30)
P value (χ^2)	<0.001 (14.050)			0.262 (1.258)	
OR (95% CI)	-			1.435 (0.762–2.700)	

Values are presented as number (%) unless otherwise indicated.

IL-1RN, interleukin-1 receptor antagonist; OR, odds ratio; CI, confidence interval; MAFLD, metabolic dysfunction-associated fatty liver disease.

Boldface indicates a statistically significant difference with $P<0.05$.

Table 5. Mean lipid profile parameters of different genotypes and alleles of ADA G22A polymorphisms among included patients (N=100)

Variable	ADA G22A genotypes		P value	IL-1RN genotypes			P value
	GG	GA		*1/*1	*1/*2	*2/*2	
Total cholesterol (mg/dL)	213 (84–220)	196.5 (79.4–219.25)	0.384	204.0 (141.0–221.5)	204.0 (75.6–220.0)	94.4 (81.0–196.3)	0.099
Triglycerides (mg/dL)	110 (69.9–118.5)	108 (82.5–117)	0.509	110.0 (75.5–113.5)	99.7 (77.5–122.5)	109.0 (99.9–117.5)	0.409
VLDL (mg/dL)	21 (17.5–23.38)	26.5 (21.75–35)	<0.001**	23.0 (20.1–34.3)	22.0 (18.8–32.0)	23.0 (20.0–25.5)	0.761
HDL (mg/dL)	31.95 (29–38.53)	35.05 (30–42.85)	0.049*	33.0 (30.0–38.6)	32.0 (28.5–40.2)	39.1 (38.9–43.4)	0.004**
VLDL/HDL ratio	0.67 (0.51–0.79)	0.75 (0.59–0.97)	0.051	0.70 (0.57–1.07)	0.75 (0.61–0.96)	0.59 (0.51–0.67)	0.056
LDL (mg/dl)	139.88 (21.9–165)	142.2 (34.33–174.1)	0.371	151.00 (60.30–173.65)	155.00 (24.53–174.10)	31.47 (22.43–133.45)	0.045*
TG/HDL-C ratio	2.93 (1.89–3.91)	2.69 (2.26–3.87)	0.955				

ADA, adenosine deaminase; IL-1RN, interleukin-1 receptor antagonist; VLDL, very low-density lipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

Boldface indicates a statistically significant difference with $P < 0.05$.

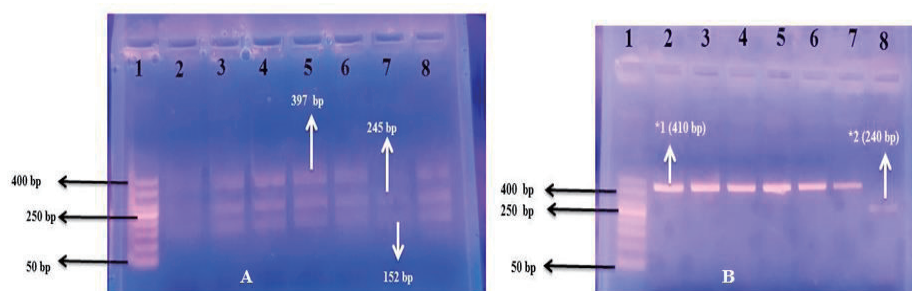


Fig. 1. ADA G22A and IL-1RN single nucleotide polymorphisms using PCR techniques. (A) Gel electrophoresis of ADA G22A polymorphism using the RFLP-PCR method; the numbers refer to lanes. Lane 1 shows the 50-bp DNA ladder; lanes 2–6 and 8 represent heterozygotes GA genotypes (397 bp, 245 bp, and 152 bp). Lane 7 represents homozygotes GG (245 bp and 152 bp). (B) Gel electrophoresis of IL-1RN polymorphism using conventional PCR method; the numbers refer to lanes. Lane 1 shows 50-bp DNA ladder, lanes 2–7 represents homozygotes *1/*1, and lane 8 represents homozygotes *2/*2. ADA, adenosine deaminase; IL-1RN, interleukin-1 receptor antagonist; RFLP-PCR, restriction fragment length polymorphism-polymerase chain reaction.

MAFLD from those without MAFLD among the included patients at cutoff value of >0.6308 with sensitivity (80%), specificity (58%), positive predictive value (PPV; 65.6%), negative predictive value (NPV; 74.4%), and accuracy (69%). TG/HDL-C ratio at cutoff value >3.0685 is a moderately effective tool (AUC=0.752) for identifying MAFLD in obese children, with good specificity (88%) and PPV (84.2%) but moderate sensitivity (64%), NPV (71%), and overall accuracy=76%.

Multivariate logistic regression analyses identified BMI, waist circumference, TG/HDL ratio, and LDL as significant predictors of MAFLD in obese children.

Discussion

Pediatric obesity is a rising global health concern, with a significant increase in prevalence among children and adolescents. The long-term consequences include type 2 diabetes, cardiovascular and respiratory diseases, and autoimmune disorders.^{16,17} While pediatric MAFLD pathogenesis remains unclear, studies suggest that obesity^{18,19}

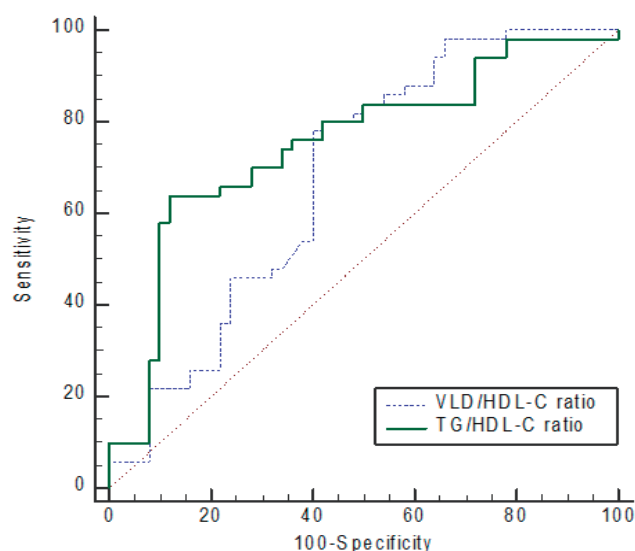


Fig. 2. Receiver operating characteristic curve showing validity of VLD-C-to-HDL-C and TG-to-HDL-C ratios at discriminating between obese children with versus without MAFLD. HDL-C, high-density lipoprotein cholesterol; MAFLD, metabolic dysfunction-associated fatty liver disease; TG, triglyceride; VLD-C, very-low-density cholesterol.

and insulin resistance^{20,21)} are key risk factors. However, susceptibility varies among children.^{22,23)}

In tandem with the rising incidence of childhood obesity, fatty liver is anticipated to grow among the leading causes of end-stage liver disease in young adults and children.²⁴⁾ The magnitude of obesity among Egyptian children in various governorates of Egypt revealed that one out of seven of 6–12 years old children in Qena governorate were obese with female predominance due to bad dietary habits with lack of physical activity.²⁵⁾ The overall prevalence of obesity among primary school children in Port Said governorate was 20.6%,²⁶⁾ while in Assiut governorate it was 12.28%,²⁷⁾ in Alexandria governorate it was 9%.²⁸⁾ A country-based study from the Eastern Mediterranean region regarding the prevalence of obesity among school aged children in Egypt revealed that the overall prevalence was 10.6%, highest in Lower Egypt (10.0%) and urban governorates (9.2%) compared to Upper Egypt governorates (6.3%).²⁹⁾ The findings of the current work revealed female sex predominance among obese children with significantly higher BMI and waist circumference among obese children particularly those with MAFLD. These findings were in agreement with previous researches.^{25–28)}

In the current study, patients had lower hemoglobin levels, higher insulin resistance, altered cortisol levels, and significant lipid profile changes compared to controls. Anemia in children may result from nutritional deficiencies or chronic diseases. Studies suggest a link between iron metabolism and lipid profiles in children. Zhu et al.²⁰⁾ observed that children with dyslipidemia had lower serum ferritin and transferrin levels, indicating a connection between iron deficiency and lipid abnormalities, especially in obese children. This suggests that iron deficiency is more prevalent in overweight or obese individuals.

Cortisol, produced by the adrenal glands, plays a crucial role in glucose metabolism and stress response. Dysregulated cortisol levels are linked to metabolic syndrome, as chronic stress elevates cortisol, leading to visceral adiposity, insulin resistance, dyslipidemia, and hypertension, highlighting the hypothalamic-pituitary-adrenal axis's role in metabolic health.^{30,31)}

A study by Chiarelli and Marcovecchio³¹⁾ found that insulin resistance in obese children is associated with hypertriglyceridemia, hypercholesterolemia, and low HDL-C. Similarly, Chang et al.³²⁾ examined inflammatory markers, lipid profiles, and insulin sensitivity, reinforcing their interconnection in pediatric obesity.

Comparing obese children with and without MAFLD reveals significant clinical and laboratory differences, emphasizing the strong link between MAFLD and meta-

bolic syndrome in children. Higher weight z scores, BMI z scores, and waist circumference in the MAFLD group align with previous studies, supporting central obesity's role in liver fat accumulation.^{33,34)}

Consistent with this study, Xing et al.³⁵⁾ reported elevated WBC counts in MAFLD patients, reinforcing the association between inflammation and metabolic disturbances. Reported also that children with MAFLD had higher fasting plasma glucose, HOMA-IR levels, ALT levels, and dyslipidemia, reinforcing the association between MAFLD and metabolic dysfunction. Insulin resistance both contributes to and results from hepatic steatosis, creating a vicious cycle that worsens metabolic dysfunction.³⁶⁾

A study on Danish children and adolescents (6–18 years old) found that overweight and obese individuals had higher ALT levels across all age groups. The study established ALT cutoff values for hepatic steatosis diagnosis at 24.5 U/L for girls and 34.5 U/L for boys.³⁷⁾ Another study using ultrasound and ALT levels to assess NAFLD found that 23.61% of overweight and obese children showed fatty liver features on ultrasound, yet 72.73% of them had normal ALT levels (<42 U/L), indicating that ALT alone may underestimate hepatic steatosis.³⁸⁾ Additionally, a study on prepubertal children with obesity reported elevated liver enzymes, leptin, insulin resistance markers, inflammation, and endothelial dysfunction, further linking obesity to metabolic disturbances.³⁹⁾

The current study observed dyslipidemia in the MAFLD group, indicating an atherogenic lipid imbalance that increases cardiovascular risk. This aligns with studies identifying dyslipidemia as a common metabolic abnormality in adolescents with metabolic syndrome.^{40–42)}

ADA plays a key role in purine metabolism, immune function, and oxidative stress regulation. The current study found that the GG genotype is significantly associated with obesity susceptibility, aligning with prior research indicating that the ADA G22A polymorphism affects immune and metabolic pathways.⁴³⁾ A study by Jadhav and Jain⁴⁴⁾ reported elevated ADA activity in overweight and obese Indian individuals, suggesting a link between increased ADA activity and obesity. Additionally, Park et al.⁴⁵⁾ investigated the A2a adenosine receptor (A2aAR) signaling in hepatic fibrosis and found that inhibiting A2aAR signaling reduced hepatic fibrosis and steatohepatitis in a mouse model, implicating adenosine pathways in liver disease progression. Given that obesity and insulin resistance are major risk factors for MAFLD, the association of the ADA G22A polymorphism with metabolic disorders such as gestational diabetes mellitus (GDM) and type 2 diabetes mellitus (T2DM) may suggest an indirect link to MAFLD.^{46,47)}

Our findings reveal a significant association between IL-1RN polymorphisms, obesity, and MAFLD in children. These results are consistent with previous research on IL-1RN polymorphisms in metabolic disorders. Strandberg et al.⁴⁸⁾ demonstrated that carriers of the IL-1RN*2 allele exhibit increased total fat, trunk fat, and serum leptin levels. Additionally, Mikhailova and Ivanoshchuk⁴⁹⁾ reported that IL-1RN, an anti-inflammatory cytokine, is secreted by white adipose tissue, with serum levels correlating positively with BMI in obese individuals, indicating a link between IL-1RN expression and obesity severity.

Knockout studies in mice suggest that the absence of the IL1RN gene confers resistance to obesity, implicating IL1RN in fat accumulation and energy balance. Moreover, Bojarczuk et al.⁵⁰⁾ highlighted that interleukin gene polymorphisms, including IL1RN, influence cytokine production and may impact energy balance, fat storage, and metabolic regulation, contributing to obesity susceptibility.

IL-1RN polymorphisms play a role in liver diseases such as MAFLD, as inflammatory cytokines, including those in the interleukin-1 family, contribute to the onset and progression of NAFLD, which shares features with MAFLD. Variations in IL-1RN may influence liver inflammation and disease progression.⁵¹⁾ A study by Maculewicz et al.¹⁾ reported that interactions between IL-1 family genes (IL1B and IL-1RN) are linked to BMI and fat percentage, with specific alleles potentially protecting against higher BMI and fat accumulation, influencing MAFLD risk.

Regarding lipid ratios and liver disease severity, Nobili et al.⁵²⁾ found that total cholesterol/HDL-C and LDL/HDL-C ratios correlate with liver injury severity in children with NAFLD, making them potential markers for disease progression.

The TG/HDL-C ratio has been explored as a marker for MAFLD in obese children. Iwani et al.⁵³⁾ associated a higher TG/HDL-C ratio with increased insulin resistance and hepatic steatosis. Similarly, Demiral et al.⁵⁴⁾ reported a significant link between TG/HDL-C ratios, insulin resistance, and hepatosteatosis in overweight and obese children. Additionally, Ting et al.⁵⁵⁾ identified TG/HDL-C ratio as an independent predictor of liver fibrosis in pediatric NAFLD, emphasizing its utility in identifying children at risk for advanced liver disease.

The relatively small sample size, evaluation of the effect of possible early lifestyle interventions with lack of long-term follow-up of the included pediatric patients with MAFLD to assess the possible outcomes were the main limitations of the current work.

Conclusion

Children with MAFLD tend to have higher weight, BMI, central obesity, and significant metabolic disturbances, including higher insulin resistance, dyslipidemia, and liver enzyme elevations. The current study highlights a potential genetic association between the ADA G22A variant and childhood obesity, while also emphasizing the significant role of the IL-1RN SNP in the development of MAFLD among obese children. Additionally, the TG-to-HDL ratio appears to be a more effective predictor of pediatric MAFLD than the VLDL/HDL ratio. Understanding these genetic influences can help in early identification of high-risk individuals and the development of targeted therapeutic approaches to prevent and manage pediatric MAFLD more effectively.

Footnotes

Conflicts of interest: No potential conflict of interest relevant to this article was reported.

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