Research Square

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Associations Among miR-33, APOE ε4, and Metabolic Markers in Down Syndrome Youth

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Abstract

Background

Down syndrome (DS), caused by trisomy 21, is associated with intellectual disability and early metabolic and neurodegenerative alterations. Cholesterol homeostasis, regulated by microRNAs (miRNAs), miR-33 through ABCA1 and HDL cholesterol (HDL-c) biogenesis, influences cognitive decline linked to the *APOE* ε4 isoform.

Methods

A cross-sectional study was conducted in 50 children and adolescents with Down syndrome (3–18 years) and matched controls, measuring miR-33a/b expression, lipid profiles, thyroid function, and APOE genotypes Statistical analyses included correlation and multivariate regression tests ($p < 0.05$).

Results

We found significantly reduced miR-33a/b levels and lower HDL-c in DS participants, alongside frequent thyroid and glycemic abnormalities. The presence of the *APOE* ε4 allele correlated with decreased HDL-c in DS, suggesting a genetic-metabolic interaction contributing to neurological vulnerability.

Conclusions

These findings suggest that dysregulation of miR-33 cknand thyroid hormones, combined with *APOE* ε4 status, may underlie early metabolic disturbances and increased neurodegenerative risk in DS, highlighting potential targets for personalized preventive and therapeutic strategies.

Registration: The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Autonomous University of Sinaloa, with registration number CEI-FM-PI-2023-009, in accordance with current ethical standards and regulations.

Background

Down syndrome (DS) is a genetic disorder resulting from an additional copy of chromosome 21, either partial or complete, leading to trisomy, characterized by intellectual disability and distinctive phenotypic features (1). Its global incidence ranges from 1 in 1,000 to 1 in 1,100 live births. In 2021, the worldwide prevalence was estimated at 19.9 per 100,000 inhabitants, corresponding to approximately 1.57 million individuals living with DS (2). Advanced maternal age (>35 years) has been identified as a major risk factor for congenital chromosomal abnormalities, including DS (1). Over the past decades, advances in prevention, diagnosis, and management of comorbidities have markedly improved the life expectancy of individuals with DS, from about 5 years in the 1950s to nearly 60 years today (3-5).

The characteristic phenotype of trisomy 21 reflects the consequences of genomic imbalance. Chromosome 21 sequencing and experimental models in humans, mice, and cell cultures have substantially contributed to understanding the molecular repercussions of this additional genetic material (6-8).

MicroRNAs (miRNAs) are key post-transcriptional regulators of gene expression and are implicated in essential biological processes, including lipid metabolism and the pathophysiology of cardiovascular and neurodegenerative diseases (9). Among them, miR-33, encoded within the introns of *SREBF2* and *SREBF1*, modulates HDL cholesterol (HDL-c) biogenesis and excretion by regulating *ABCA1* expression as well as bile acid synthesis and secretion (9, 10). In animal models, inhibition of miR-33 elevates plasma HDL-c levels and confers protection against atherosclerosis (11).

Regulated expressions of miR-33 may therefore exert protective effects against atherosclerosis and other HDL-c related disorders, representing a potential therapeutic target (11). In individuals with DS, no direct association or specific phenotypic role of miR-33 has been reported. However, given its established role in cholesterol metabolism via *ABCA1* regulation and HDL biogenesis, mechanisms also implicated in cognitive decline linked to the *APOE* ε4 isoform, its potential contribution in DS warrants investigation (10, 12).

Evidence from animal and human models indicates that miR-33 regulates lipid transport and oxidation pathways by interacting with genes such as *ABCA1* and *SREBP*, thereby modulating HDL biogenesis and cholesterol flux at the tissue level. Moreover, the functional connection between miR-33 and brain lipid-regulatory systems underscores the integration of miRNA signaling with proteins such as *APOE* and transporters, including ABCA1, in maintaining cerebral lipid homeostasis and neuroprotection. Dysregulation of miR-33 and APOE may therefore jointly impair cholesterol metabolism and β-amyloid processing, offering novel therapeutic and diagnostic perspectives for neurodegenerative diseases associated with HDL-c (13, 14).

Plasma HDL-c levels and the function of lipoproteins such as *APOE* are essential for vascular integrity, cerebral cholesterol balance, and β-amyloid clearance. Variants of the APOE gene, particularly ε4, have been linked to increased risk of cognitive decline and dementia, especially in individuals with DS, where they accelerate the onset of Alzheimer's disease, like symptoms, and disrupt cerebral lipid homeostasis (15).

Functionally, *APOE* regulates cerebral cholesterol metabolism, including β-amyloid clearance, through interactions with ABCA1 and lipoprotein receptors (16). It is also a well-established genetic determinant of neurodegenerative diseases such as Alzheimer's, which manifests earlier in individuals with DS (17, 18). The ε4 isoform has been consistently associated with an increased risk and faster progression of cognitive impairment, whereas ε2 appears to confer a protective effect (17, 19). In people with DS, the ε4 allele is associated with more severe, earlier-onset cognitive decline (19).

Thyroid dysfunction is also markedly more prevalent among individuals with DS, occurring at significantly higher rates than in the general population, from childhood through adulthood. These disorders tend to appear earlier and have a pronounced impact on neurological development. Combined with genetic factors such as *APOE* ε4, thyroid dysfunction may potentiate or accelerate cognitive deterioration in this population (20).

Given the high prevalence of dyslipidemias characterized by low HDL-c levels, frequent thyroid abnormalities, and the genetic predisposition conferred by APOE variants in DS, along with the regulatory role of miR-33 in lipid metabolism, it is essential to investigate the interactions among these factors. Their combined influence may contribute to early cognitive decline in DS (9, 17, 21). Understanding these mechanisms could help identify specific biomarkers and therapeutic targets to mitigate cognitive deterioration and cardiovascular comorbidities in this population.

Methods

Study design

A cross-sectional study with a descriptive, correlational design was conducted to compare individuals with DS with healthy control participants (CTRL). Written informed consent was obtained from the parents or legal guardians of all participants. All collected data were anonymized to ensure confidentiality. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Autonomous University of Sinaloa, with registration number CEI-FM-PI-2023-009, in accordance with current ethical standards and regulations.

Study population

Initially, 91 individuals with a confirmed diagnosis of DS were recruited through an open social media call. Eligible participants included individuals with a history of thyroid disorders or prior surgical procedures. Exclusion criteria included a previous diagnosis of leukemia, incomplete clinical information, or insufficient blood sample for analysis. After applying inclusion and exclusion criteria, the final study population comprised 50 participants with cytogenetically confirmed DS, aged 3 to 18 years, of both sexes (Figure 1). Additionally, a control group of 50 healthy participants without DS or other known genetic conditions, matched by age and sex, was also enrolled.

Anthropometric evaluation

Body weight and body fat percentage were measured using a digital scale (Tanita BF689, Tokyo, JP). Height was measured using a stadiometer (SECA 2013, Birmingham, UK), and waist and hip circumferences were measured using a metallic tape (Lufkin, Apex Tool Group, Sparks, MD, USA). All measurements were taken with participants barefoot and wearing light clothing, following standardized procedures established by the International Society for the Advancement of Kinanthropometry (ISAK) (22). Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). BMI in the DS group was classified using age-appropriate percentile tables from the National Institute of Pediatrics (INP): <5^{th} percentile, underweight; 5^{th}–90^{th}, healthy weight; 90^{th}–95^{th}, overweight, and >95^{th}, obesity (23). For the CTRL group, BMI-for-age percentiles were determined according to the Centers for Disease Control and Prevention (CDC): <5^{th} percentile, underweight; 5^{th}–85^{th}, healthy weight; 85^{th}–95^{th}, overweight; and >95^{th}, obesity (24).

Biochemical evaluation

Peripheral blood was collected via venipuncture after 10-12 hours of overnight fasting into EDTA and SST tubes, centrifuged at 3500 rpm for 10 min, and stored at −80°C until analysis. Serum concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), and HDL-c were determined using an enzymatic colorimetric method (RANDOX Laboratories Ltd., Antrim, UK). Dyslipidemia was defined according to the National Cholesterol Education Program (NCEP) criteria for children and adolescents: TC ≥ 200 mg/dL; TG ≥ 100 mg/dL (0–9 years) or ≥130 mg/dL (≥10 years); HDL-c < 40 mg/dL; and LDL-c ≥ 130 mg/dL (25). Atherogenic risk was estimated using the TC/HDL-c ratio and the logarithmic TG/HDL-c index (26). Glucose and glycated hemoglobin (HbA1c) were measured by enzymatic colorimetry (RANDOX Laboratories Ltd., Antrim, UK) and classified according to the American Diabetes Association (ADA) criteria: glucose ≥126 mg/dL and HbA1c ≥6.5% as thresholds for diabetes risk.

MiRNA quantification

Plasma miRNAs were extracted using the phenol-based TRIzol reagent (Invitrogen Life Technologies) from 200 µL of plasma per participant. Extracted RNA was diluted in 8 µL of RNase-free water and stored at −80°C. cDNA synthesis was performed with the miRCURY™ LNA™ RT Kit (QIAGEN Cat. No. 339340) in a 10 µL reaction containing 7 µL of purified RNA. Reaction conditions were as follows: reverse transcription at 45°C for 60 min, inactivation at 95°C for 5 min, and storage at 4°C. Quantification of miR-33a-5p (miR-33a) and miR-33b-3p (miR-33b) expression was performed by real-time quantitative PCR (qPCR) using the miRCURY LNA SYBR® Green PCR Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. *SNORD48* was used as the endogenous control (12, 27). Reactions were run on a StepOnePlus™ Real-Time PCR System (Applied Biosystems) under the following conditions: 95°C for 2 min; 40 cycles of 95°C for 10 s; and melt curve stage of 95°C for 15 s, 60°C for 1 min, and a gradual temperature increase of 0.3°C per step, and a final denaturation at 95°C for 15 seconds, during which fluorescence data were continuously collected. PCR efficiencies were calculated using LinRegPCR software (28).

APOE genotyping

Genomic DNA was extracted from blood leukocytes using the DNeasy Blood & Tissue Kit (QIAGEN, Cat. 69504, Hilden, Germany). Allelic discrimination of *APOE* isoforms was performed by real-time PCR using TaqMan® probes (29) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). The analyzed variants were rs429358 and rs7412, located in exon 4 of the APOE gene, which together determine the ε2, ε3, and ε4 alleles. Genotype analysis was performed using TaqMan Genotyper Software (30, 31).

Statistical analysis

Normality of data distribution was assessed using the Kolmogorov–Smirnov test. Continuous variables are presented as mean ± standard deviation (SD). Between-group comparisons were conducted using paired or unpaired Student's *t*-tests for parametric data, and Mann–Whitney or Wilcoxon tests for

nonparametric data. Categorical variables were analyzed using the chi-square test (χ^2). Measures of central tendency and dispersion were calculated for all quantitative variables. Pearson or Spearman correlation coefficients were applied depending on data distribution, and multiple linear regression analyses were performed to assess associations between HDL-c and key anthropometric and biochemical parameters. Statistical analyses were conducted using GraphPad Prism 8.0 and SPSS version 25.0, with $p < 0.05$ considered significant.

Results

Study population

The study included 50 individuals with DS (DS group) and 50 age- and sex-matched individuals without DS (CTRL group). Sixty-two percent of the participants were male, with ages ranging from 3 to 18 years (mean age of 10.0 ± 3.7 years). In the DS group, karyotype analysis revealed that 96% of participants had full trisomy 21, 2% had a translocation, and another 2% exhibited mosaicism (Table 1).

Table 1. General characteristics of the study groups

Variable	CTRL group n (%)	DS group n (%)
Age (years)	10.0 ± 3.7	10.0 ± 3.7
Sex		
Male	31 (62.0)	31 (62.0)
Female	19 (38.0)	19 (38.0)
Karyotype		
Full trisomy 21	-	48 (96.0)
Translocation	-	1 (2.0)
Mosaicism	-	1 (2.0)

Values are presented as mean ± standard deviation (SD). CTRL: control; DS: Down syndrome.

Anthropometric characteristics

Participants with DS showed significantly lower mean body weight and height compared to controls (34.4 ± 15.6 vs. 41.9 ± 21.9 kg, $p = 0.0028$; 125.1 ± 17.7 vs. 141.0 ± 20.1 cm, $p = 0.0001$, respectively). No significant differences were observed in BMI, waist, or hip circumference between groups (Table 2).

Table 2. Anthropometric characteristics of the study groups

Variable	**CTRL group** Mean ± SD	**DS group** Mean ± SD	***p value***
Weight (kg)	41.9 ± 21.9	34.4 ± 15.6	**0.0028**
Heigh (cm)	141.0 ± 20.1	125.1 ± 17.7	**0.0001**
BMI (kg/m²)	20.8 ± 5.3	19.7 ± 6.1	0.1053
Waist (cm)	65.8 ± 13.9	65.0 ± 15.3	0.8905
Hip (cm)	77.4 ± 17.1	75.4 ± 14.8	0.2959
%Fat (%)	22.8 ± 9.3	24.8 ± 9.3	0.4446

CTRL: control; DS: Down syndrome; BMI: body mass index; %Fat: body fat percentage. $p < 0.05$ was considered statistically significant.

Although BMI did not differ significantly, a higher proportion of DS participants have overweight or obesity (38%) compared to controls (24%), while the proportion with a healthy weight was greater in the CTRL group (70% vs. 60%).

Biochemical characteristics

Biochemical analysis revealed that individuals with DS had significantly lower HDL-c concentrations than controls (47.5 ± 13.3 vs. 55.5 ± 13.7 mg/dL, $p = 0.0047$) and higher TG levels (97.6 ± 38.4 vs. 75.9 ± 36.0 mg/dL, *p = 0.0139*). TC did not differ significantly; however, the TC/HDL-c ratio was higher in the DS group (3.6 ± 2.2 vs. 2.9 ± 0.6, $p = 0.0181$).

The plasma atherogenic index (PAI) was also elevated in DS ($p = 0.0007$), suggesting increased cardiovascular risk. Thyroid function parameters (TSH, free and total T4, total T3) showed significant differences between groups (Table 3).

Table 3. Biochemical concentrations in the study groups

Variable	CTRL group Mean ± SD	DS group Mean ± SD	*p value*
TC (mg/dL)	160.0 ± 32.4	155.8 ± 31.4	0.5369
TG (mg/dL)	75.9 ± 36.0	97.6 ± 38.4	**0.0139**
HDL-c (mg/dL)	55.5 ± 13.7	47.3 ± 13.3	**0.0047**
LDL-c (mg/dL)	89.2 ± 21.5	88.8 ± 26.9	0.9391
VLDL-c (mg/dL)	14.6 ± 6.3	19.9 ± 8.6	**0.0035**
TC/HDL-c	2.9 ± 0.6	3.6 ± 2.2	**0.0181**
IAP [Log(TG/HDL-c)]	0.1 ± 0.2	0.2 ± 0.2	**0.0007**
Glucose (mg/dL)	92.1 ± 14.8	92.6 ± 7.7	0.4850
HbA1c (%)	5.1 ± 0.3	5.1 ± 0.4	0.6431
Uric acid (mg/dL)	3.9 ± 1.1	5.2 ± 0.9	**0.0001**
Urea (mg/dL)	21.1 ± 7.1	27.9 ± 6.7	**0.0001**
Creatinine (mg/dL)	0.4 ± 0.1	0.5 ± 0.1	**0.0068**
TSH (mg/dL)	2.4 ± 1.5	3.8 ± 2.3	**0.001**
Free T4 (mg/dL)	1.1 ± 0.1	1.2 ± 0.2	**0.0134**
Total T4 (mg/dL)	7.7 ± 1.6	8.7 ± 2.0	**0.0043**
Free T3 (mg/dL)	4.6 ± 1.0	4.8 ± 0.7	0.3699
Total T3 (mg/dL)	142.8 ± 30.6	122.8 ± 26.5	**0.0036**
miR-33a	1.5 ± 1.2	0.9 ± 1.9	**0.0047**
miR-33b	1.6 ± 1.4	133.4 ± 727.0 (0.6 ± 1.6)	0.5043

CTRL: control; DS: Down syndrome; TC: total cholesterol; TG: Triglycerides; HDL-c: HDL cholesterol; LDL-c: LDL cholesterol; VLDL-c: VLDL cholesterol; IAP: plasma atherogenic index; HbA1c: glycated hemoglobin; TSH: thyroid-stimulating hormone; T4: thyroxine; T3: triiodothyronine; IAP: plasma atherogenic index. $p < 0.05$ was considered statistically significant.

The DS group showed a greater frequency of dyslipidemias (hypertriglyceridemia, low HDL-c, and high LDL-c) and thyroid dysfunction compared to controls, although not all reached statistical significance. The atherogenic risk was significantly higher in DS ($p = 0.0001$), with 66% classified as high risk versus 30% in the CTRL group (Table 4).

Table 4. Categorical classification of variables of interest

Variable	CTRL (n=50)	DS (n=50)	*p value*
BMI			**0.0156**
Underweight (%)	6	2	
Normal weight (%)	70	60	
Overweight/Obesity (%)	24	38	
Dyslipidemias			0.0631
Hypercholesterolemia (%)	12	10	
Hypertriglyceridemia (%)	14	28	
Low HDL-c (%)	12	24	
High LDL-c (%)	2	8	
Thyroid disorders (%)			**0.0001**
Hypothyroidism (%)	0	48	
Hyperthyroidism (%)	0	4	
Low/High TSH (%)	6	34	
Low/High free T4 (%)	2	2	
Low/High total T4 (%)	4	4	
Low/High free T3 (%)	73	58	
Low/High total T3 (%)	16	6	
Atherogeneity			**0.0001**
Low risk (%)	52	22	
Medium risk (%)	16	12	
High risk (%)	30	66	

The values represent percentages (%). CTRL: control, DS: Down syndrome, BMI: Body mass index, HDL-c: HDL cholesterol, LDL-c: LDL cholesterol, TSH: thyroid-stimulating hormone, T4: thyroxine, T3: triiodothyronine. $p < 0.05$ was considered significant.

Expression levels of miR-33a and miR-33b

Expression of both miR-33a and miR-33b was significantly lower in the DS group compared with controls ($p < 0.0001$ for boths). (Figure 1a). Median relative expression was 0.044 vs. 1.077 for for miR-33a and 0.145 vs. 1.322 for miR-33b (Figure 2a and 2b).

Age-stratified analysis (3–11 and 12–18 years) confirmed these trends. In both age groups, miR-33a expression remained lower in DS. For miR-33b, expression was also reduced in younger DS participants (Figure 3a) but showed a relative increase in the 12–18 years subgroup (Figure 3b).

APOE genotyping

APOE genotyping was performed in 30 DS and 40 CTRL samples. The ε3/ε3 genotype was the most frequent (DS: 73.6%; CTRL: 67.5%). The ε4/ε4 genotype was rare (2.6% in both groups). The ε4 allele frequency was similar (DS: 11.8%, CTRL: 12.5%) (Table 5).

Table 5. APOE genotypic and allelic frequencies

Genotype frequency					Allele frequency				
	CTRL		DS			CTRL		DS	
Genotype	**n**	**%**	**n**	**%**	**Allele**	**n**	**%**	**n**	**%**
ε2/ε2	0	0.0	1	2.6	ε2	4	5.0	5	6.6
ε2/ε3	4	10.0	1	2.6	ε3	66	82.5	62	81.6
ε2/ε4	0	0	2	5.6	ε4	10	12.5	9	11.8
ε3/ε3	27	67.5	28	73.6	**Total**	80	100	76	100
ε3/ε4	8	20.0	5	13.0					
ε4/ε4	1	2.5	1	2.6					
Total	40	100	38	100					

The values represent the number of individuals with each genotype, the total number of alleles (n), and the percentage (%). CTRL: control, DS: Down syndrome, *APOE*: apolipoprotein E.

In carriers of the ε4/ε4 genotype, HDL-c concentrations were lower in DS (43.0 mg/dL) than in controls (63.5 mg/dL, $p = 0.0002$) (Figure 4). When grouped by ε4 allele carriage, the mean HDL-c was lower in the at-risk DS subgroup (41.1 mg/dL) compared to the non-risk subgroup (48.4 mg/dL). In contrast, controls carrying ε4 showed higher HDL-c (61.0 mg/dL) than non-carriers (50.7 mg/dL) (Figure 5).

miR-33a and miR-33b expression were also influenced by *APOE* genotype. DS individuals carrying the ε4 risk allele exhibited higher expression of miR-33a (1.567 vs. 0.701) and miR-33b (2.170 vs. 0.585) than non-carriers. These differences were less pronounced in controls (Figure 6b).

Correlation of variables of interest

In the DS group, free T3 was positively correlated with body fat percentage ($r = 0.580$, $p = 0.002$), while total T4 was negatively correlated with HDL-c ($r = -0.331$, $p = 0.021$). HbA1c correlated negatively with TC ($r = -0.280$, $p = 0.049$) and positively with HDL-c ($r = 0.405$, $p = 0.004$) and creatinine ($r = 0.479$, $p < 0.001$). miR-33a correlated positively with TSH ($r = 0.318$, $p = 0.036$), and negatively with free T3 ($r = -0.349$, $p = 0.040$). miR-33b correlated positively with TC ($r = 0.298$, $p = 0.040$) and negatively with waist circumference ($r = -0.348$, $p = 0.040$) (Table 6).

In contrast, in controls, total T3 and free T4 correlated negatively with TC ($r = -0.341$, $p = 0.016$; $r = -0.318$, $p = 0.026$, respectively). HbA1c showed negative correlations with HDL-c ($r = -0.372$, $p = 0.009$) and total T4 ($r = -0.382$, $p = 0.007$), and a positive correlation with the atherogenic index ($r = 0.341$, $p = 0.016$) (Table 7).

Table 6. Correlation analysis between anthropometric and biochemical variables and miR-33 in the DS group.

Variable			1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	**Age (years)**	*r*	1													
		p														
2	**Height (cm)**	*r*	.928**	1												
		p	0.000													
3	**Weight (kg)**	*r*	.901**	.882**	1											
		p	0.000	0.000												
4	**%Fat (%)**	*r*	-0.145	-0.168	0.356	1										
		p	0.452	0.383	0.058											
5	**Waist (cm)**	*r*	.644**	.612**	.720**	0.315	1									
		p	0.000	0.000	0.000	0.096										
6	**Hip (cm)**	*r*	.839**	.815**	.969**	.502**	.743**	1								
		p	0.000	0.000	0.000	0.006	0.000									
7	**CT (mg/dL)**	*r*	-0.154	-0.155	-0.082	0.241	-0.052	-0.106	1							
		p	0.284	0.284	0.573	0.207	0.723	0.467								
8	**TG (mg/dL)**	*r*	0.155	0.128	0.214	0.316	0.236	0.204	.537**	1						
		p	0.281	0.376	0.135	0.095	0.102	0.159	0.000							
9	**HDL-c (mg/dL)**	*r*	0.116	0.173	0.105	-0.134	0.034	0.047	0.207	-0.245	1					
		p	0.421	0.230	0.468	0.487	0.818	0.747	0.150	0.086						
10	**LDL-c (mg/dL)**	*r*	-0.259	-.283*	-0.193	0.256	-0.124	-0.190	.901**	.452**	-0.176	1				
		p	0.070	0.047	0.179	0.180	0.394	0.192	0.000	0.001	0.221					
11	**TC-HDL-c**	*r*	-0.038	0.025	0.030	0.072	0.129	0.058	-0.127	0.104	-0.259	-0.048	1			
		p	0.795	0.864	0.834	0.712	0.378	0.694	0.378	0.471	0.069	0.740				
12	**Atherogenic risk**	*r*	-0.096	-0.076	-0.031	0.271	0.141	0.016	0.150	0.213	-0.160	0.191	.760**	1		
		p	0.509	0.602	0.829	0.155	0.334	0.911	0.300	0.137	0.269	0.183	0.000			
13	**TSH (mg/dL)**	*r*	-.300*	-0.240	-0.256	-0.039	-0.180	-0.223	0.065	-0.008	0.064	0.018	0.082	0.158	1	
		p	0.043	0.109	0.086	0.844	0.238	0.141	0.667	0.956	0.675	0.907	0.587	0.296		
14	**Free T3 (mg/dL)**	*r*	0.036	0.148	0.218	.580**	0.017	0.230	0.148	0.002	0.284	0.020	-0.295	-0.061	-0.146	1
		p	0.835	0.391	0.201	0.002	0.923	0.183	0.389	0.992	0.094	0.906	0.081	0.724	0.394	
15	**Total T3 (mg/dL)**	*r*	-0.329	-0.236	-0.300	0.148	-0.254	-0.271	0.240	-0.172	0.159	0.224	-0.003	0.102	-0.066	.5
		p	0.050	0.166	0.076	0.462	0.141	0.115	0.158	0.316	0.353	0.189	0.985	0.555	0.704	0.
16	**Free T4 (mg/dL)**	*r*	-0.088	-0.174	-0.084	0.080	-0.005	-0.112	0.019	0.128	-0.146	0.072	0.040	-0.020	-0.040	-.3
		p	0.546	0.232	0.566	0.685	0.971	0.450	0.897	0.382	0.318	0.625	0.785	0.892	0.791	0.
17	**Total T4 (mg/dL)**	*r*	-0.067	-0.067	-0.064	-0.047	0.022	-0.078	0.093	0.198	-.331*	0.200	-0.021	-0.081	-0.072	-0
		p	0.650	0.649	0.666	0.815	0.884	0.601	0.530	0.176	0.021	0.174	0.889	0.583	0.636	0.
18	**Creatinine (mg/dL)**	*r*	.801**	.752**	.757**	-0.194	.456**	.722**	-0.138	0.148	0.169	-0.278	-0.106	-0.175	-0.248	0.
		p	0.000	0.000	0.000	0.312	0.001	0.000	0.340	0.306	0.240	0.050	0.464	0.223	0.097	0.
19	**Urea (mg/dL)**	*r*	0.015	0.002	0.005	-0.119	0.150	0.017	-0.004	-0.099	0.156	-0.064	-0.021	-0.141	-0.097	-0
		p	0.917	0.992	0.970	0.539	0.303	0.908	0.981	0.496	0.280	0.657	0.883	0.327	0.519	0.
20	**Uric acid (mg/dL)**	*r*	.506**	.469**	.576**	0.016	.391**	.511**	-0.057	0.224	0.111	-0.183	-0.211	-0.173	-0.256	0.
		p	0.000	0.001	0.000	0.934	0.008	0.000	0.706	0.134	0.462	0.223	0.159	0.250	0.098	0.
21	**HbA1c (%)**	*r*	.521**	.505**	.515**	-0.172	.487**	.480**	-.280*	-0.244	.405**	-.454**	0.072	-0.074	-0.136	0.
		p	0.000	0.000	0.000	0.372	0.000	0.000	0.049	0.087	0.004	0.001	0.618	0.609	0.367	0.
22	**miR-33a**	*r*	-0.172	-0.176	-0.220	-0.267	-0.197	-0.239	-0.098	-0.251	-0.259	-0.069	-0.024	-0.215	.318*	-.3

		p	0.243	0.231	0.132	0.170	0.184	0.106	0.506	0.085	0.076	0.641	0.874	0.142	0.036	0.
23	**miR-33b**	*r*	-0.139	-0.184	-0.227	-0.349	-.348*	-0.265	.298*	-0.109	0.060	.346*	0.113	0.210	0.056	-0
		p	0.347	0.211	0.120	0.068	0.017	0.071	0.040	0.462	0.684	0.016	0.444	0.152	0.717	0.

The values represent the correlation coefficient (r). %Fat: body fat percentage; TC: total cholesterol; TG: Triglycerides; HDL-c: HDL cholesterol; LDL-c: LDL cholesterol; TC-HDL-c: total cholesterol/HDL cholesterol ratio; TSH: thyroid-stimulating hormone; T4: thyroxine; T3: triiodothyronine; HbA1c: glycated hemoglobin A1c; miR-33a: miRNA-33a; miR-33b: miRNA-33b. *$p < 0.05$, **$p < 0.01$.

Table 7. Correlation analysis between anthropometric and biochemical variables and miR-33 in the CTRL group.

Variable			1	2	3	4	5	6	7	8	9	10	11	12	13
1	**Age (years)**	*r*	1												
		p													
2	**Height (cm)**	*r*	.923**	1											
		p	0.000												
3	**Weight (kg)**	*r*	.781**	.881**	1										
		p	0.000	0.000											
4	**%Fat (%)**	*r*	0.100	0.018	.428**	1									
		p	0.498	0.903	0.002										
5	**Waist (cm)**	*r*	.596**	.557**	.734**	.720**	1								
		p	0.000	0.000	0.000	0.000									
6	**Hip (cm)**	*r*	.789**	.765**	.708**	.523**	.814**	1							
		p	0.000	0.000	0.000	0.000	0.000								
7	**CT (mg/dL)**	*r*	0.022	0.038	0.052	0.053	-0.061	-0.031	1						
		p	0.880	0.801	0.726	0.719	0.679	0.833							
8	**TG (mg/dL)**	*r*	0.188	0.139	0.278	.488**	0.249	0.234	.531**	1					
		p	0.196	0.351	0.056	0.000	0.088	0.110	0.000						
9	**HDL-c (mg/dL)**	*r*	-0.113	-0.044	-0.154	-0.217	-0.215	-0.175	.666**	0.059	1				
		p	0.438	0.768	0.296	0.139	0.142	0.234	0.000	0.688					
10	**LDL-c (mg/dL)**	*r*	0.057	0.051	0.115	0.097	-0.029	0.002	.905**	.428**	.346*	1			
		p	0.696	0.734	0.438	0.511	0.845	0.992	0.000	0.002	0.015				
11	**TC-HDL-c**	*r*	0.218	0.137	.329*	.410**	.313*	.285*	0.262	.531**	-.519**	.547**	1		
		p	0.133	0.359	0.022	0.004	0.030	0.050	0.069	0.000	0.000	0.000			
12	**Atherogenic risk**	*r*	0.210	0.136	.316*	.485**	.358*	.296*	0.161	.848**	-.394**	0.211	.705**	1	
		p	0.147	0.364	0.029	0.000	0.012	0.041	0.268	0.000	0.005	0.146	1.551		
13	**TSH (mg/dL)**	*r*	-.505**	-.522**	-.435**	0.013	-0.272	-.435**	-0.023	0.024	-0.003	-0.022	-0.052	-0.004	1
		p	0.000	0.000	0.002	0.931	0.062	0.002	0.873	0.872	0.982	0.880	0.721	0.978	
14	**Free T3 (mg/dL)**	*r*	-.440**	-.413**	-0.133	0.152	-0.127	-.291*	-.341*	-0.169	-.282*	-0.278	-0.019	0.005	0.137
		p	0.002	0.004	0.369	0.303	0.389	0.045	0.016	0.247	0.050	0.053	0.898	0.974	0.347
15	**Total T3 (mg/dL)**	*r*	-.576**	-.535**	-0.176	-0.007	-0.244	-.508**	-0.194	-0.064	-0.191	-0.150	0.014	0.039	0.175
		p	0.000	0.000	0.231	0.960	0.095	0.000	0.182	0.660	0.190	0.304	0.923	0.789	0.228
16	**Free T4 (mg/dL)**	*r*	-0.021	-0.059	0.005	-0.075	0.015	0.032	-.318*	-0.192	-0.129	-.332*	-0.244	-0.145	0.078
		p	0.884	0.694	0.973	0.614	0.918	0.830	0.026	0.187	0.376	0.020	0.092	0.320	0.592
17	**Total T4 (mg/dL)**	*r*	-.289*	-.295*	0.044	.317*	0.163	0.000	-0.192	-0.193	-0.124	-0.145	-0.126	-0.111	.314*
		p	0.044	0.044	0.766	0.028	0.269	0.998	0.186	0.183	0.398	0.319	0.388	0.447	0.028
18	**Creatinine (mg/dL)**	*r*	.703**	.775**	.558**	-0.066	.398**	.572**	.371**	0.230	0.193	.360*	0.204	0.146	-0.254
		p	0.000	0.000	0.000	0.656	0.005	0.000	0.009	0.112	0.184	0.011	0.160	0.316	0.078
19	**Urea (mg/dL)**	*r*	-0.065	-0.021	-0.150	-0.205	-0.103	-0.128	0.213	-0.001	0.149	0.226	0.031	-0.081	0.166
		p	0.655	0.888	0.309	0.162	0.486	0.388	0.141	0.997	0.307	0.118	0.832	0.578	0.254
20	**Uric acid (mg/dL)**	*r*	.413**	.507**	.564**	0.231	.571**	.485**	0.117	0.187	0.043	0.086	0.152	0.195	-0.148
		p	0.003	0.000	0.000	0.114	0.000	0.000	0.424	0.198	0.768	0.556	0.297	0.179	0.310
21	**HbA1c (%)**	*r*	-0.036	0.004	0.054	-0.017	-0.072	-0.029	0.227	.300*	-0.049	0.273	.372**	.341*	-0.151
		p	0.805	0.979	0.715	0.908	0.625	0.846	0.116	0.036	0.740	0.057	0.009	0.016	0.299
22	**miR-33a**	*r*	0.075	0.199	0.217	0.014	0.040	0.047	0.007	-0.024	-0.125	0.126	0.053	0.079	0.168

		p	0.694	0.309	0.259	0.941	0.836	0.807	0.971	0.898	0.510	0.507	0.780	0.680	0.376
23	**miR-33b**	*r*	-0.001	0.130	0.252	0.029	0.172	0.120	0.062	0.038	-0.151	0.122	0.208	0.106	0.234
		p	0.994	0.511	0.187	0.883	0.372	0.535	0.746	0.842	0.425	0.522	0.271	0.576	0.214

The values represent the correlation coefficient (r). %Fat: body fat percentage; TC: total cholesterol; TG: Triglycerides; HDL-c: HDL cholesterol; LDL-c: LDL cholesterol; TC-HDL-c: total cholesterol/HDL cholesterol ratio; TSH: thyroid-stimulating hormone; T4: thyroxine; T3: triiodothyronine; HbA1c: glycated hemoglobin A1c; miR-33a: miRNA-33a; miR33-b: miRNA-33b. *$p < 0.05$, **$p < 0.01$.

Multiple linear regression

In the DS group, multiple regression analysis identified HbA1c as a positive predictor of HDL-c (β = 13.25; 95% CI: 2.53 to 23.9; $p = 0.017$), whereas total T4 was negatively associated with HDL-c (β = –2.47; 95% CI: –4.92 to –0.02; $p = 0.048$). No other variables showed significant associations. In the CTRL group, none of the analyzed variables were significantly associated with HDL-c (Table 8).

Table 8. Results of multiple linear regression between HDL-c and metabolic variables

	HDL-c (CTRL)		**HDL-c (DS)**	
	β (IC 95%)	***p value***	**β (IC 95%)**	***p value***
HbA1c (%)	-2.39 (-16.57 to 11.77)	0.735	13.25 (2.53 to 23.9)	**0.017**
TSH (mg/dL)	0.217 (-2.60 to 3.04)	0.877	1.81 (-0.25 to 3.88)	0.084
Free T4 (mg/dL)	-10.69 (-48.29 to 26.90)	0.569	8.28 (-12.06 to 28.64)	0.412
Total T4 (mg/dL)	0.22 (-3.25 to 3.71)	0.896	-2.47 (-4.92 to -0.02)	**0.048**
Free T3 (mg/dL)	-3.96 (-10.02 to 2.10)	0.194	3.49 (-4.15 to 11.14)	0.358
Total T3 (mg/dL)	0.01 (-0.19 to 0.19)	0.967	0.09 (-0.11 to 0.29)	0.366

Dependent variable: HDL-c. Values represent the regression coefficient (β), 95% confidence intervals (95% CI), and P-values. HDL-c: high-density lipoprotein cholesterol, HbA1c: glycated hemoglobin, TSH: thyroid-stimulating hormone, T4: thyroxine, T3: triiodothyronine.

Discussion

In this study, we quantified miR-33a and miR-33b expression, HDL-c concentrations, and the frequency of the *APOE* ε4 genotype in children and adolescents with DS compared with control subjects. The results showed significantly lower expression of both miRNAs in the DS group, accompanied by an unfavorable lipid profile.

Individuals with DS had markedly lower mean body weight and height compared with controls (34.4 ± 15.6 kg and 125.1 ± 17.7 cm vs. 40.9 ± 21.9 kg and 141.0 ± 20.1 cm, respectively). This pattern is attributed to hypothalamic–pituitary axis dysfunctions and reduced growth hormone levels (32-35). A higher prevalence of overweight and obesity was observed in DS (38%) compared with controls (25%), consistent with previous reports describing frequent excess weight and its association with metabolic alterations in this population (36, 37).

This relationship has been partially explained by the interplay of genetic, hormonal, and behavioral factors that predispose individuals with DS to increased adiposity and dyslipidemia (38). Nevertheless, prevalence estimates vary widely among studies. For instance, one study reported only 15% of participants with DS had overweight or obesity, yet identified a significant association between BMI and triglyceride concentrations (39). This supports the notion that even moderate increases in adiposity contribute substantially to dyslipidemia in DS.

Regarding thyroid function and metabolic alterations, our findings align with recent evidence showing higher rates of thyroid dysfunction and metabolic abnormalities in pediatric DS populations (40). DS participants in our study exhibited significantly higher TSH, total and free T4, and total T3 concentrations than controls, suggesting both central and peripheral dysregulation of thyroid function (20). These alterations may contribute to increased susceptibility to metabolic syndrome and disruptions in glucose and lipid metabolism (41).

An adverse lipid profile was observed in DS, characterized by lower HDL-c levels and higher TG concentrations, findings consistent with previous studies in DS, including comparisons with unaffected siblings (33, 42, 43). The elevated atherogenic index observed here underscores a higher cardiovascular risk in this group.

Altered lipid metabolism may be modulated by dysregulation of miR-33a and miR-33b, known regulators of cholesterol efflux and HDL formation (11). Interestingly, the decreased expression of miR-33 observed in our DS cohort contrasts with studies reporting miR-33 overexpression in dyslipidemic models (11, 14). This discrepancy may stem from methodological differences, particularly the biological matrix analyzed (plasma vs. tissues), as miRNA expression can vary markedly across sample types (44). Subject age and specific genetic and metabolic features of pediatric DS populations, including obesity, may also influence miR-33 expression (45).

Unlike previous work examining other miRNAs such as miR-155 and let-7c Pérez-Villarreal, Aviña-Padilla (34), we did not identify significant correlations between miR-33 levels and other measured parameters in controls. The younger age of our participants (3–18 years) may partially explain this difference, suggesting that miR-33 expression could increase with age (34). Overall, these findings indicate that miR-33 regulation is dynamic during development, warranting further investigation to elucidate its role in DS pathophysiology.

The presence of the *APOE* ε4 allele, an established risk factor for Alzheimer's disease, was associated with lower HDL-c levels in DS, despite similar allele frequencies between groups. This suggests that the impact of ε4 on lipid metabolism may be modulated by the DS genetic background (46). Specifically, DS individuals carrying the ε4/ε4 genotype exhibited markedly lower HDL-c levels than controls with the same genotype (41.1 vs. 61.0 mg/dL, $p = 0.0002$). This reinforces the hypothesis that gene-environment interactions in DS amplify metabolic and neurological vulnerability (46, 47).

Thyroid hormones may modulate the relationship between lipid metabolism and neurodegenerative processes in DS. The positive correlation between free T3 and body fat percentage, and the inverse correlation between total T4 and HDL-c, suggest altered enzymatic regulation within the T3/T4 axis (48). These changes could impair HDL-mediated cholesterol transport, including eflux from brain tissue, potentially compromising neuronal lipid homeostasis.

Given that individuals with DS are prone to autoimmune thyroid dysfunction and early neurodegeneration, thyroid dysregulation may intensify chronic inflammation and disrupt central cholesterol balance. Reduced HDL-c concentrations could impair *APOE*-mediated lipid transport and clearance, thereby heightening neurodegenerative risk (49). Collectively, these mechanisms highlight a complex interplay between thyroid dysfunction, lipid imbalance, and neuronal vulnerability in DS. Understanding these interactions could guide preventive interventions targeting metabolic pathways before irreversible neurodegenerative changes occur.

Regression analysis revealed interactions between glycemic and thyroid axes: HbA1c was positively associated with HDL-c ($\beta = 13.25$; $p = 0.017$), while total T4 was negatively associated ($\beta = -2.47$; $p = 0.048$). These results suggest that better glycemic control may enhance HDL-c concentrations and offer neuroprotective benefits, whereas thyroid alterations characteristic of DS could contribute to HDL-c depletion and increased Alzheimer's risk.

Interestingly, HbA1c correlated negatively with TC but positively with HDL-c in DS, in contrast with the atherogenic pattern typically observed in the general population (50). This atypical metabolic phenotype may reflect DS-specific compensatory mechanisms, altered insulin signaling, or distinctive regulation of reverse cholesterol transport.

Recent studies support this interpretation, showing that stable HbA1c levels are associated with reduced dementia risk, whereas hyperthyroidism increases susceptibility to Alzheimer's disease (51, 52).

Although similar HbA1c-lipid relationships have been reported in DS (41), our findings provide stronger statistical evidence for this association.

The positive correlation between miR-33a and TSH, and its negative correlation with free T3, suggest potential thyroid regulation activity of miR-33a in DS. Although several miRNAs are expressed in thyroid tissue (53), no prior studies have reported such association for miR-33. These findings raise the possibility that miR-33a modulates thyroid-axis-mediated metabolic responses, potentially by regulating genes involved in lipid metabolism (54).

The positive correlation between miR-33a and TC, together with its negative correlation with waist circumference, further supports a potential role in adipose redistribution and lipid regulation. Experimental studies in murine DS model have demonstrated tissue-dependent miR-33 expression, with elevated levels in muscle, adipose, and hepatic tissues compared to controls, where expression predominates in adipose tissue (14). Collectively, these findings support the hypothesis that miR-33a may serve as a molecular mediator linking thyroid function, lipid regulation, and body composition in DS.

While associations between thyroid hormones and lipids observed in controls reflect typical physiological regulation, in DS these relationships extend to anthropometric and miRNA variables, underscoring a more complex metabolic network. A recent study reported differences in thyroid hormone sensitivity and its associations with metabolic parameters in euthyroid DS children and adolescents compared with controls (40).

To our knowledge, this is the first study to simultaneously evaluate miR-33 expression and *APOE* genotyping in relation to HDL-c and thyroid hormones in a pediatric DS population. By integrating molecular, biochemical, and genetic markers, we reveal novel interactions among miR-33, *APOE* ε4, and thyroid hormones, providing insight into mechanisms linking metabolic dysfunction to neurological vulnerability. These findings suggest the potential value of early metabolic monitoring and genetic screening strategies in DS, with potential implications for anticipating and preventing neurodegenerative outcomes.

These findings suggest the potential value of early metabolic monitoring and genetic screening strategies in DS, with potential implications for anticipating and preventing neurodegenerative outcomes.

LIMITATIONS

This study has several limitations that should be acknowledged when interpreting the results. The moderate sample size and cross-sectional design limit the ability to establish causal relationships or assess the temporal progression of the observed metabolic and molecular alterations. Moreover, the exclusive assessment of plasma miR-33 expression restricts a comprehensive understanding of its biological behavior, as its levels may vary across tissues and cellular compartments. This underscores the importance of incorporating tissue-based analyses or cellular models in future research to clarify its specific functional role in DS.

Another relevant limitation is the low frequency of the *APOE* ε4 allele in the study cohort, which constrains the statistical power to draw robust conclusions about its genetic impact in this pediatric population. Additionally, age-related variations may influence miR-33 expression.

Despite these limitations, the present findings provide a valuable foundation for longitudinal investigations of the metabolic and genetic axes in DS. Future studies should explore the temporal evolution of miR-33 and *APOE* ε4 in relation to lipid homeostasis, thyroid function, and neurodegenerative risk. Expanding the cohort size will strengthen the generalizability of these results and help define the integrative role of these biomarkers in the metabolic and cognitive health of individuals with DS.

Conclusions

The findings of this study demonstrate that children and adolescents with Ds exhibit a distinctive metabolic profile characterized by lower expression of miR-33a and miR-33b, reduced HDL-c concentrations, and concomitant thyroid and glycemic alterations. Collectively, these features are associated with metabolic and neurodegenerative risk. The positive correlation between HbA1c and HDL-c, together with the negative association between total T4 and HDL-c, suggests a complex interplay among glucose, lipid, and thyroid regulatory pathways in this population, distinct from the pattern observed in individuals without DS. The association between the *APOE* ε4 allele and lower HDL-c levels in the DS group further supports a functional interaction between cholesterol transport and neurodegenerative mechanisms linked to Alzheimer's disease. This reinforces the concept that early metabolic disturbances are associated with neurological vulnerability in individuals with DS.

Overall, these results support the hypothesis of altered regulation of cholesterol homeostasis mediated by miR-33 dysregulation and thyroid dysfunction in the context of trisomy 21. They also highlight the importance of further investigating the interaction between miRNAs, genetic risk factors such as *APOE* ε4, and metabolic pathways connecting lipid metabolism with neurodegeneration. These findings raise the possibility that the identified molecular and metabolic alterations could serve as early biomarkers, which may informe future research on personalized preventive and therapeutic strategies to reduce neurodegenerative risk and improve quality of life in the DS population.

Abbreviations

The following abbreviations are used in this manuscript:

DS: Down syndrome

CTRL: Control

miRNAs: MicroRNAs

miR-33a: miR-33a-5p

miR-33b: miR-33b-3p

APOE: Apoliporproteín E

HDL-c: High-density lipoprotein cholesterol

TC: Total cholesterol

TG: Triglycerides

LDL-c: Low-density lipoprotein cholesterol

VLDL-c: Very low-density lipoprotein cholesterol

Glu: Glucose

HbA1c: Glycated hemoglobin

AIP: Atherogenic index of plasma

TSH: Thyroid function parameters

T4: Thyroxine

T3: Triiodothyronine

Declarations

Ethics approval and consent to participate: The study received approval from the Research Ethics Committee of the Faculty of Medicine, Autonomous University of Sinaloa (registration number CONBIOÉTICA-25-CEI-003-20181012), and was conducted in accordance with the ethical principles established in the Declaration of Helsinki. A written informed consent was obtained from the parent or legal guardian of each participant.

Consent for publication: All data were anonymized to protect participant confidentiality.

Availability of data and materials: The datasets supporting the findings of this study can be obtained from the principal author (keniaesparza.fcqb@uas.edu.mx), upon reasonable request. However, the data is not publicly accessible as it includes information that may compromise the confidentiality of the research participants.

Competing interests: The authors declare no competing interests.

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Figures

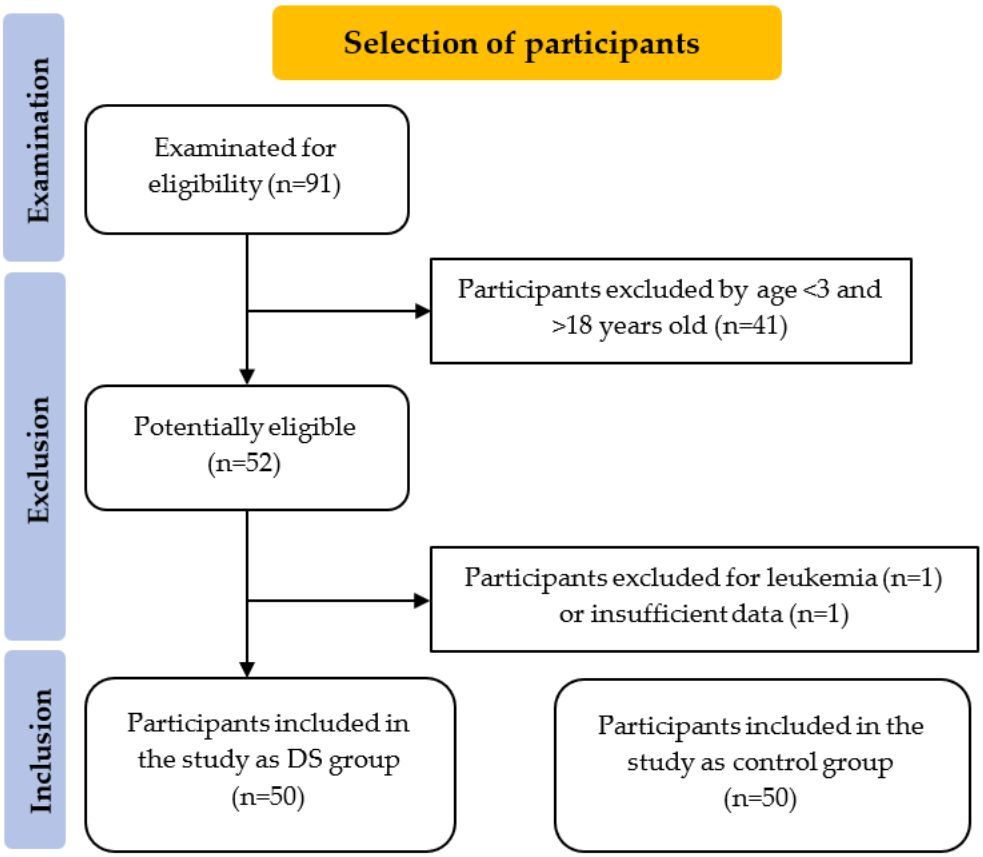


Figure 1

Population of the study

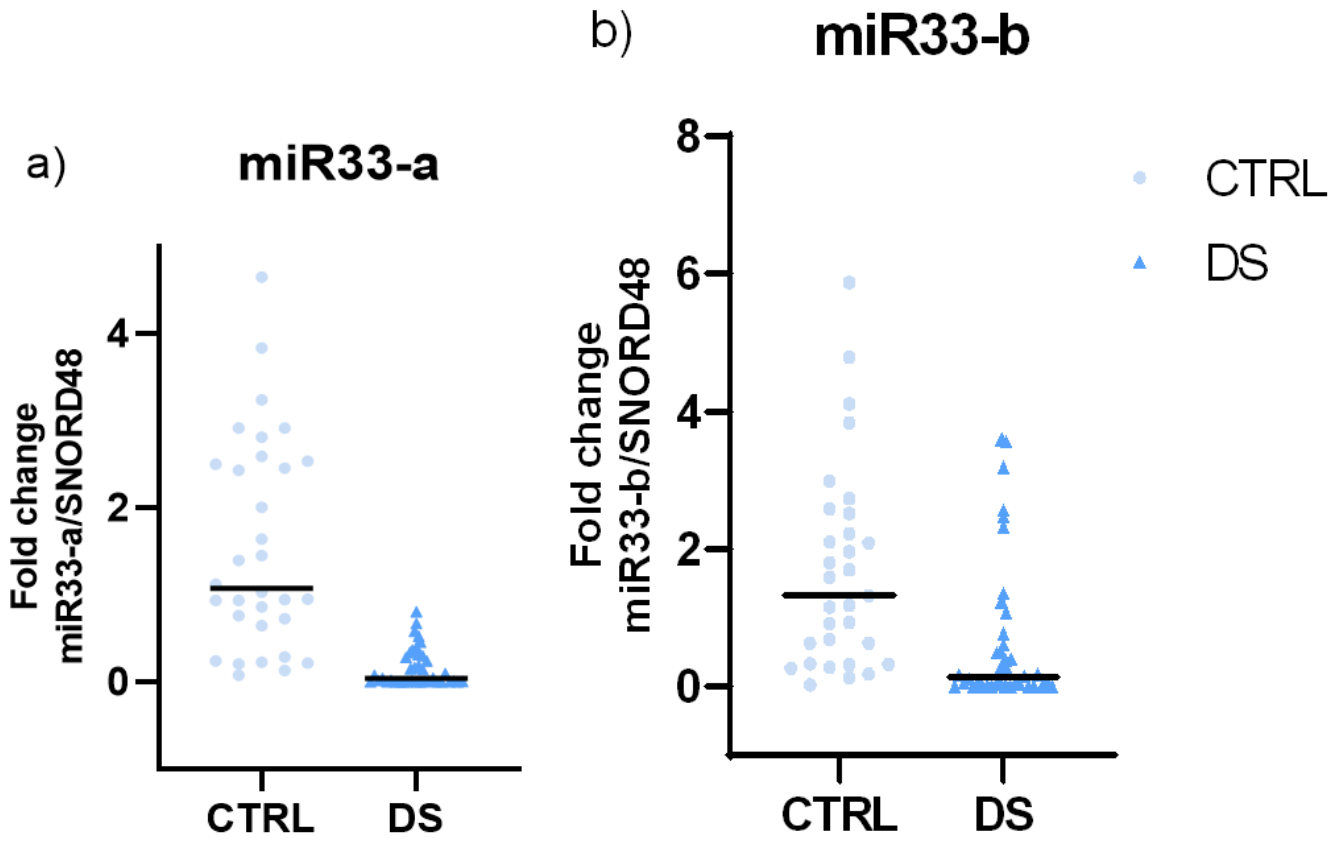


Figure 2

Expression levels of a) miR-33a and b) miR-33b in the study groups.

miR-33a: miRNA-33a, miR-33b: miRNA-33b, SNORD48: small nucleolar RNA 48, CTRL: control, DS: Down syndrome.

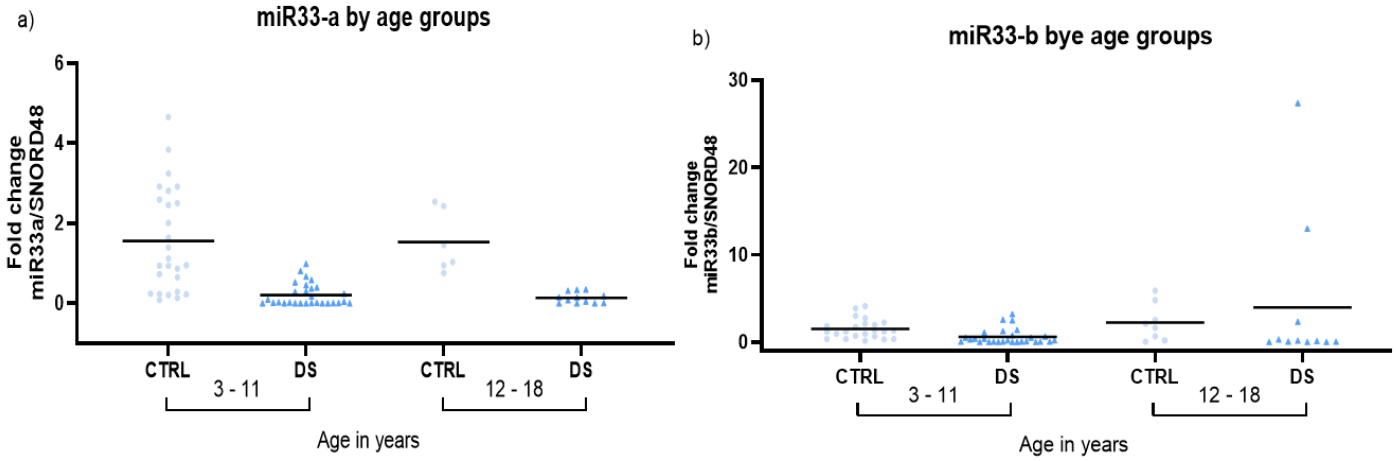


Figure 3

Expression of a) miR-33a and b) miR-33b by age groups in the study cohorts.

CTRL: control, DS: Down syndrome.

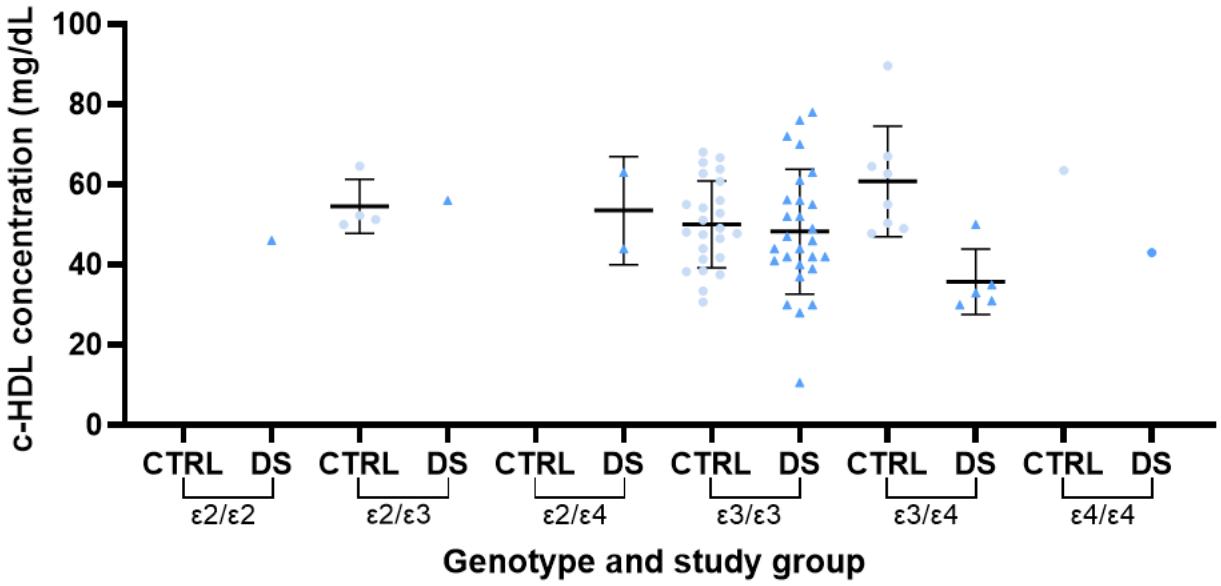


Figure 4

Genotype and HDL-c concentration in the study groups.

Values represent the mean concentration of the study groups. HDL-c: HDL cholesterol; CTRL: control; DS: Down syndrome; ε: apolipoprotein E epsilon.

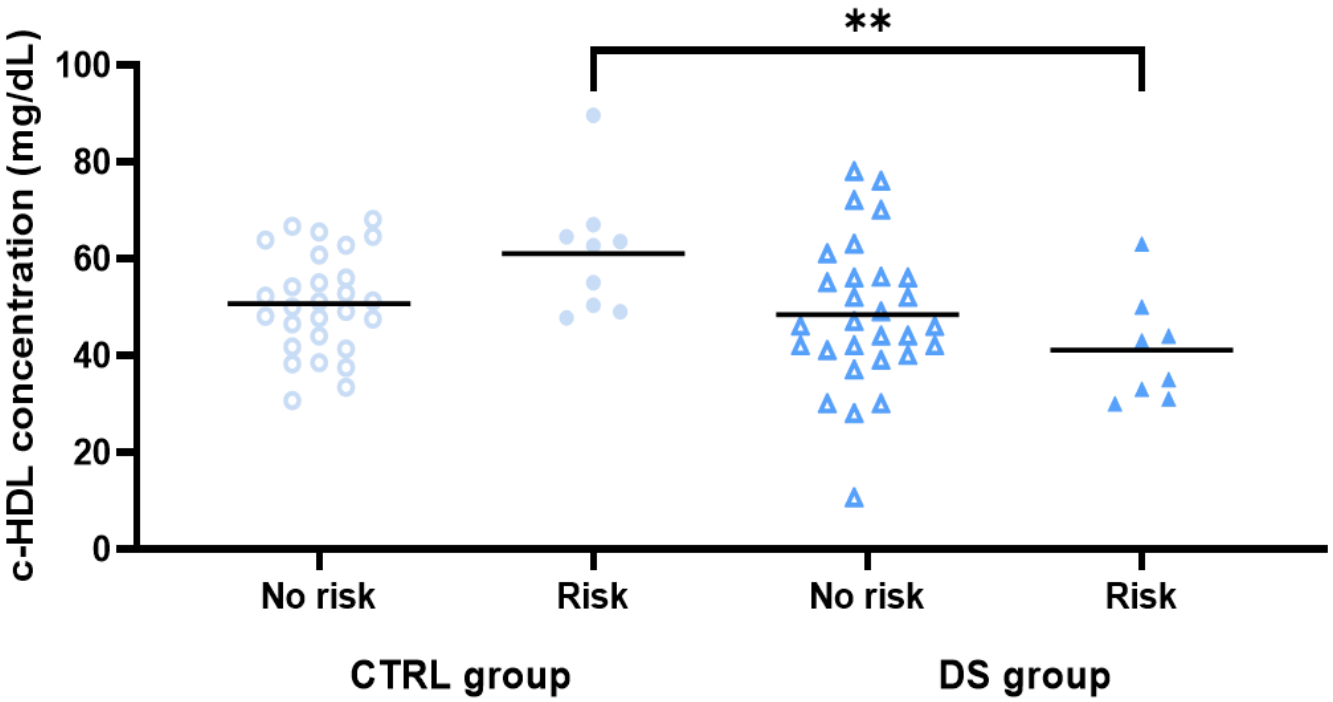


Figure 5

Risk allele and HDL-c in the study groups.

Data points represent HDL-c concentrations in the study groups, stratified by the presence of the *APOE* risk allele ε4. HDL-c: HDL cholesterol; CTRL: control; DS: Down syndrome; Risk: presence of the apolipoprotein E ε4 allele, $^{**}p < 0.01$.

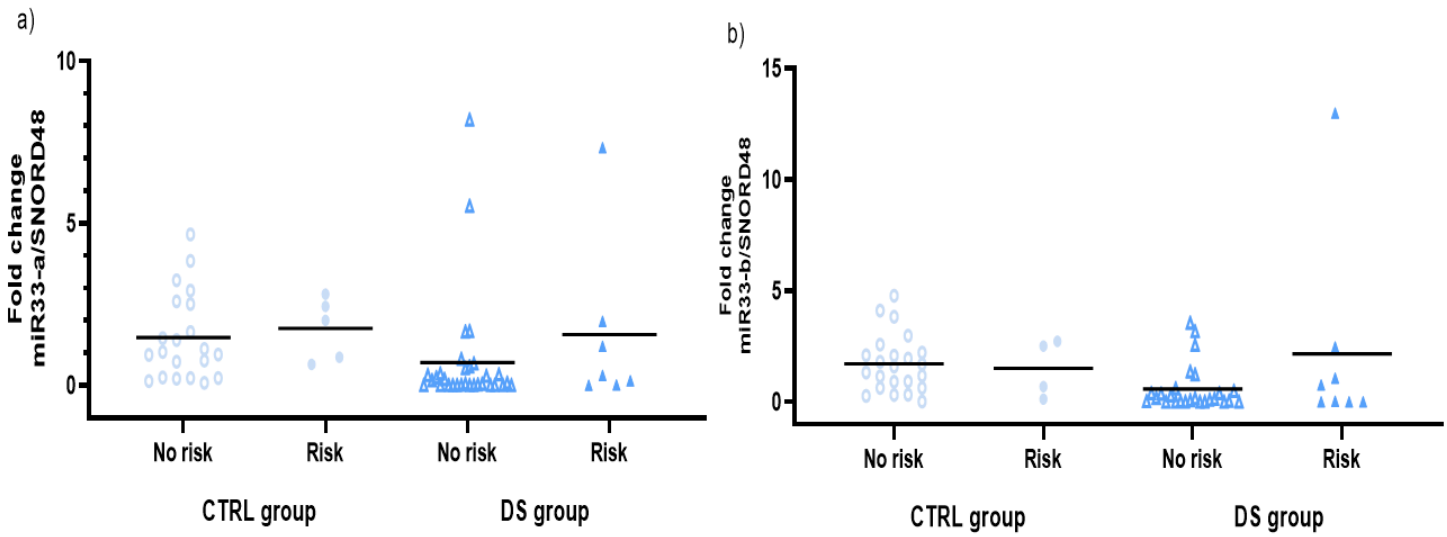


Figure 6

Risk allele and expression of miR-33a and miR-33b in the study groups.

Dots represent the expression levels of miR-33a and miR-33b in the study groups, stratified by the presence of the *APOE* risk allele. miR-33a: miRNA-33a, miR33-b: miRNA-33b, SNORD48: small nucleolar RNA 48, CTRL: control, DS: Down syndrome, Risk: presence of the *APOE* risk allele ε4.