RESEARCH ARTICLE

Trends of *LSS* rs2254524, *LSS* rs2254522, and *NEDD4L* rs4149601 Genetic Variants of Hypertension Among Obese Adolescents

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Abstract

BACKGROUND: Polymorphisms in genes regulating renal sodium handling, such as lanosterol synthase (*LSS*) and neural precursor cell expressed developmentally down-regulated 4-like (*NEDD4L*), as well as the urinary sodium-to-potassium (Na/K) ratio have been proposed as determinants of blood pressure among obese adolescents, but evidence on gene-environment interactions remains limited. This study was conducted to examine associations between *LSS* (rs2254524) and *NEDD4L* (rs4149601) polymorphisms and the urinary Na/K ratio and hypertension in obese adolescents.
METHODS: This cross-sectional comparative study enrolled 202 obese adolescents (101 hypertensive, 101 normotensive) aged 15–18 years old. Genotyping was performed by polymerase chain reaction (PCR) followed by Sanger sequencing. An ion-selective electrode was used to measure the spot urinary sodium and potassium for the calculation of Na/K ratio. Associations of the gene polymorphism and urinary sodium and potassium interaction were then statistically analyzed.
RESULTS: Male gender, body weight, height, body mass index (BMI), and waist circumference were significantly higher in the hypertensive group (all $p<0.0001$). Though hypertension was numerically more common among obese adolescents carrying *LSS* variants (rs2254524, rs2254522) and *NEDD4L* variant (rs4149601), neither *LSS* (A>C, A>G) nor *NEDD4L* genotypes were significantly associated with hypertension in bivariate analyses (all $p>0.05$). The urinary Na/K ratio did not differ between groups ($p=0.889$).
CONCLUSION: Genetic variants of *LSS* rs2254524, *LSS* rs2254522, and *NEDD4L* rs4149601 are showing trends of hypertension among obese adolescents in this study. Greater adiposity and male gender, by contrast, were significantly associated with hypertension and remained the dominant correlates of blood pressure.
KEYWORDS: *LSS, NEDD4L,* polymorphism, sodium-potassium ratio, hypertension, obesity, adolescent, gene-environment interaction

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Introduction

Childhood obesity has reached epidemic proportions globally, with prevalence among adolescents increasing more than threefold since 1975.(1) Obesity during adolescence predisposes individuals to numerous cardiometabolic complications, with hypertension being one of the most important comorbidities.(2) Other pediatric metabolic risk factors, including childhood hyperuricemia, have also been associated with a higher risk of future hypertension,(3) and cardiovascular gene polymorphisms have been linked to blood pressure regulation and vascular pathology.(4–6) Pooled epidemiological studies indicate that the

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prevalence of hypertension among obese adolescents was higher (15.27%) compared to overweight (4.99%) and normoweight (1.90%).(7) The pathogenesis of obesity-related hypertension involves complex interactions between genetic susceptibility and environmental factors, including dietary sodium and potassium intake.(8)

The urinary sodium-to-potassium (Na/K) ratio has emerged as an integrated biomarker that reflects the balance between sodium and potassium intake and has been proposed as a more reliable predictor of blood pressure than either electrolyte measured in isolation.(9) Studies in adult populations have demonstrated that the urinary Na/K ratio correlates with blood pressure levels and cardiovascular outcomes.(10) In adolescent populations, the spot urinary Na/K ratio has been associated with blood pressure levels in healthy adolescents, suggesting its utility for assessing salt loading and blood pressure elevation.(11)

Genetic variation plays a significant role in modulating blood pressure responses to dietary electrolytes. The lanosterol synthase (*LSS*) gene is known as a key enzyme in steroid biosynthesis, and its variant rs2254524 (Val642Leu) missense mutation has been linked to salt sensitivity in humans.(12) Patients carrying the A allele of *LSS* rs2254524 show greater blood pressure reduction in response to low salt intake, with only CC genotype patients experiencing increases in plasma endogenous ouabain (EO) during salt restriction.(13) The *LSS* AA variant impacts blood pressure when a high-salt diet is consumed, while LSS gene polymorphisms affect both salt sensitivity of blood pressure and alterations in EO circulation in response to low salt intake.(13,14)

The neural precursor cell expresses the developmentally down-regulated 4-like (*NEDD4L*) gene, which, when expressed in neural precursor cells, encodes an E3 ubiquitin ligase that controls epithelial sodium channel (ENaC) expression in the distal nephron.(15) The functional SNP rs4149601 (G/A) influences *NEDD4L*'s ability to regulate ENaC expression and is associated with high blood pressure and salt sensitivity.(16) G allele carriers of this SNP show higher salt sensitivity and greater blood pressure response to salt intake compared to AA genotype patients.(17) *NEDD4L* polymorphisms influence the variability in response to a low-salt diet among individuals with essential hypertension, with patients carrying the AA genotype experiencing a more significant reduction in systolic blood pressure.(18)

Despite these findings in adult populations, research examining the relationship between *LSS* and *NEDD4L* polymorphisms and urinary electrolyte balance in obese adolescents remains limited. Obese individuals may exhibit distinct electrolyte handling patterns due to alterations in renal function, insulin sensitivity, and neurohormonal regulation.(19–21) This study was conducted to examine associations between *LSS* rs2254524 and *NEDD4L* rs4149601 polymorphisms and urinary Na/K ratio in obese adolescents with and without hypertension.

Methods

Study Design and Participants

This cross-sectional study was carried out at several high schools in Padang, Indonesia, between February and June 2026, involving 202 obese adolescents aged 15–18 years, with an equal split of 101 hypertensive and 101 normotensive individuals. The sample size was calculated using the Hypothesis Test for the Difference in Proportions Between Two Groups equation, with a 10% dropout rate. Obesity was defined as a body mass index (BMI) above the 95th percentile for age and sex, according to the Centers for Disease Control and Prevention (CDC) 2000 Growth Charts.(22) Hypertension was defined as elevated systolic and/or diastolic blood pressure above 130/80 mmHg, consistent with the 2017 American Academy of Pediatrics Clinical Practice Guideline.(23)

Subjects with a history of acute or chronic kidney disease, acute or chronic illnesses affecting fluid-electrolyte balance, or currently using antihypertensive medications or medications affecting sodium/potassium excretion were excluded from the study. All subjects and their parents provided written informed consent and assent. The recruitment of the subjects was performed according to the ethically approved study protocol.

Clinical and Anthropometric Data Collection

Blood pressure was measured using a cuff-oscillometric OMRON HBP-1320 (Omron Corporation, Kyoto, Japan) upper-arm monitor following standardized procedures. Anthropometric data including body weight, height, BMI, waist circumference, and waist-to-height ratio (WtHR) were collected by a clinician using a standard technique.

Urinary Sodium and Potassium Levels Analysis

Spot urine samples were taken from the second morning fasting void for the measurement of urinary sodium and potassium levels. Urinary sodium and potassium concentrations were measured using the ion-selective electrode (ISE) method on an ARCHITECT c8000 automated electrolyte/chemistry analyzer (Abbott

Laboratories, Abbott Park, IL, USA), which was calibrated with the manufacturer's standard solutions before each analytical run and underwent daily internal quality control. The urinary Na/K ratio for each participant was calculated by dividing the urinary sodium concentration (mmol/L) by the urinary potassium concentration (mmol/L).

PCR and Sanger Genotyping

DNA was extracted from peripheral blood or saliva samples using the GeneJet Whole Blood Genomic DNA Purification Mini Kit (Cat. No. K0722; Thermo Fisher Scientific, Waltham, MA, USA). *LSS* rs2254524 and *NEDD4L* rs4149601 genotypes were determined using PCR followed by Sanger sequencing. Primer sequences were: LSS-F1 (5'-TGGTTCACTTCTAAGAGGCGA-3') and LSS-R1 (5'-GTCAAGCTCGGGTTTCAGTA-3') for *LSS*; NEDD4L-F1 (5'-ATCTTATGTGGCGTTATCTGGG-3') and NEDD4L-R1 (5'-AGGCCTGGTTAGACTTGTTCC-3') for *NEDD4L*. These PCR products were sequenced at 1stBASE Malaysia, and sequences were analyzed using multiple alignment against reference sequences (NCBI NG_011510.1 for LSS; NCBI NG_029954.1 for NEDD4L). The sequencing of the *LSS* amplicon, which primarily targeted rs2254524, also captured the adjacent variant rs2254522, which falls within the amplified region.

Statistical Analysis

Data analysis was conducted using IBM SPSS Statistics 25 (IBM Corp., Armonk, New York, USA). Data distribution was first assessed with the Kolmogorov-Smirnov test. Depending on this distribution, continuous variables were presented as mean±standard deviation (SD) or median (interquartile range), while categorical variables were expressed as frequencies and percentages. Between-group comparisons used the independent t-test, Mann-Whitney U test, or chi-square test, as appropriate, with $p<0.05$ and alpha of 5% considered statistically significant. A Cochran-Armitage trend test was used to evaluate the dose-response relationship between the number of variant alleles and hypertension prevalence for each genotype (*LSS* rs2254524, *LSS* rs2254522, and *NEDD4L* rs4149601).

Results

Subjects Characteristics

The proportion of males was higher in the hypertension group than in the normotension group (64.9% *vs.* 35.1%, $p<0.0001$). Body weight, height, BMI percentile, and waist circumference were significantly higher in the hypertension group ($p<0.0001$ for all variables), as were systolic and diastolic blood pressure, consistent with the group definitions. The WtHR did not differ significantly between groups ($p=0.072$). Similarly, no significant differences were found between groups in age, ethnicity, urinary sodium levels, urinary potassium levels, or the Na/K ratio (all $p>0.05$) (Table 1).

Genotype Distribution and Its Association with Hypertension

Mean urinary Na/K ratio across on *LSS* and *NEDD4L* genotype are presented in Table 2. For the *LSS* rs2254524 (A>C) polymorphism, the CC genotype was more common in adolescents with hypertension (58.3%) than the AC (45.5%) and AA (44.4%) genotypes (Table 3); notably, hypertension prevalence was similar between AA and AC (1.1 percentage-point difference) and rose mainly in the CC genotype, indicating the numerical trend was concentrated in CC homozygotes rather than increasing stepwise with each additional C allele. In contrast, the AA and AC genotypes were more common in the normotensive group. This distribution was not statistically significant ($p=0.2110$; comparison of wild-type *vs*. variant in Table 4: $p=0.6210$). This variant showed a significant deviation from Hardy–Weinberg equilibrium in the normotensive group ($p=0.0090$), but not in the hypertensive group ($p=0.1480$). For the *LSS* rs2254522 (A>G) polymorphism, the AG (54.8%) and GG (54.2%) genotypes were more common in adolescents with hypertension, whereas the AA genotype was more prevalent in the normotensive group (58.1%). This association did not reach statistical significance (Table 3, $p=0.2150$; wild-type *vs.* variant, Table 4, $p=0.0800$). For the *NEDD4L* rs4149601 polymorphism, the GA genotype was more common in the hypertension group (60.4%), while the GG (52.8%) and AA (58.3%) genotypes were more common in the normotension group. No significant association with hypertension was found (Table 3, $p=0.2380$; wild-type *vs.* variant, Table 4, $p=0.2180$). This variant showed a significant deviation from Hardy–Weinberg equilibrium in the normotensive group ($p=0.0017$), but not in the hypertensive group ($p=0.4300$) (Table 5).

To assess whether hypertension proportion changed systematically with increasing variant-allele dose, Cochran-Armitage trend test was applied to each variant, with genotypes ordered by the number of variant alleles (0, 1, or 2). The resulting *p*-trend indicated the null hypothesis of no linear association between allele dose and hypertension proportion; it was independent of the reference category

Table 1. Demographic and clinical characteristics of study participants.

Variable	Hypertension (n=101)	Normotension (n=101)	*p*-value
Gender, n (%)			
Male	72 (64.9)	39 (35.1)	<0.0001[a,*]
Female	29 (31.9)	62 (68.1)	
Ethnicity, n (%)			
Minangkabau	87 (51.8)	81 (48.2)	0.2590[a]
Non-Minangkabau	14 (41.2)	20 (58.8)	
Age (years), median (min-max)	16.8 (15.10-18.9)	16.8 (15.1-19)	0.6880[c]
Body weight (kg), median (min-max)	91.3 (70.6-136.3)	82 (63.1-128)	<0.0001[c,*]
Height (cm), median (min-max)	165.1 (132.9-180)	158 (145-185)	<0.0001[c,*]
BMI percentile, median (min-max)	98.38 (94.18-99.98)	97.38 (93.57-99.62)	<0.0001[b,*]
Systolic (mmHg), mean±SD	134.28±8.47	112.12±6.29	<0.0001[b,*]
Diastolic (mmHg), mean±SD	83.88±6.82	71.87±4.64	<0.0001[c,*]
Waist circumference (cm), median (min-max)	99 (80-141)	95 (80-122)	<0.0001[c,*]
Waist-to-height ratio, median (min-max)	0.6 (0.48-1.04)	0.59 (0.5-0.71)	0.0720[c]
Urinary sodium (mmol/L), mean±SD	168.8±63.60	173.02±71.32	0.6200[b]
Urinary potassium (mmol/L), mean±SD	54.28±25.20	53.87±26.4	0.9100[b]
Urinary sodium-to-potassium ratio, median (min-max)	3.45 (0.69-10.83)	3.32 (0.44-12.32)	0.8890[c]

[a]Chi-square test used for categorical variables; [b]independent t-test used for normally distributed continuous variables; [c]Mann-Whitney U test used for non-normally distributed continuous variables. The percentage was calculated by row.

used for the odds ratios (Table 6). None of the three variants showed a statistically significant linear trend: *LSS* rs2254524 (44.4% → 45.5% → 58.3% across AA, AC, CC; *p*-trend=0.1080), *LSS* rs2254522 (41.9% → 54.8% → 54.2% across AA, AG, GG; *p*-trend=0.1310), and *NEDD4L* rs4149601 (47.2% → 60.4% → 41.7% across GG, GA, AA; *p*-trend=0.4750). The non-monotonic pattern for *NEDD4L* rs4149601 in particular argues against a true dose-response relationship for this variant in the present sample.

Overall, the three polymorphisms (*LSS* rs2254524, *LSS* rs2254522, *NEDD4L* rs4149601) showed no statistically significant association with hypertension status in obese adolescents, either in comparisons based on genotype (Table 3), in comparisons between the wild-type and variant alleles (Table 4), or in the genotype dose-response trend analysis (Table 6).

Table 2. Mean urinary Na/K ratio based on genotype and hypertension status.

Variant	Genotype	Na/K	
		Hypertension	Normotension
LSS A>C	AA	3.68±1.78	3.76±2.09
	AC	3.72±2.28	4.21±2.43
	CC	3.96±2.26	3.23±1.53
LSS A>G	AA	3.67±2.11	4.29±2.44
	AG	3.99±2.25	3.54±2.04
	GG	2.30±2.46	2.66±1.60
NEDD4L	GG	3.85±2.11	3.90±2.26
	GA	3.86±2.54	3.61±2.25
	AA	3.18±2.04	4.27±1.23

Gene–Electrolyte Interaction Analysis

Based on Table 7, no significant interaction was found between the *LSS* rs2254524 genotype and urinary sodium levels (variant: *p*=0.4000; wild-type: *p*=0.2430) or urinary potassium levels (variant: *p*=0.7070; wild-type: *p*=0.2100) with respect to blood pressure. Similarly, no significant interactions were found between the *LSS* rs2254522 genotype and sodium (variant: *p*=0.4250; wild-type: *p*=0.7190) or potassium (variant: *p*=0.1920; wild-type: *p*=0.1170), nor between the *NEDD4L* genotype and sodium (variant: *p*=0.9750; wild-type: *p*=0.6290) or potassium (variant: *p*=0.3960; wild-type: *p*=0.7240). All 95% confidence intervals for these interactions included the value 1, indicating that neither urinary sodium nor

Table 3. Association of *LSS* and *NEDD4L* gene polymorphisms with hypertension incidence in obese adolescents (by individual genotype).

Variant	Genotype	n (%)			OR	95% CI	*p*-value	*p*-trend
		Hypertension	Normotension	Total				
LSS A>C	AA	8 (44.4)	10 (55.6)	18 (100)	0.957	0.352–2.56	0.2110[a]	0.1080[b]
	AC	51 (45.5)	61 (54.5)	112 (100)	0.571	0.202–1.61		
	CC	42 (58.3)	30 (41.7)	72 (100)				
	Total	101 (50.0)	101 (50.0)	202 (100)				
LSS A>G	AA	31 (41.9)	43 (58.1)	74 (100)	0.594	0.326–1.085	0.2150[a]	0.1310[b]
	AG	57 (54.8)	47 (45.2)	104 (100)	0.61	0.242–1.54		
	GG	13 (54.2)	11 (45.8)	24 (100)				
	Total	101 (50.0)	101 (50.0)	202 (100)				
NEDD4L	GG	67 (47.2)	75 (52.8)	142 (100)	0.585	0.3–1.13	0.2380[a]	0.4750[b]
	GA	29 (60.4)	19 (39.6)	48 (100)	1.251	0.37–4.12		
	AA	5 (41.7)	7 (58.3)	12 (100)				
	Total	101 (50.0)	101 (50.0)	202 (100)				

Reference category for each variant is the last-listed genotype (CC for *LSS* A>C; GG for *LSS* A>G; AA for *NEDD4L*). [a]Tested with Chi-square test. [b]Tested with Cochran–Armitage trend test.

potassium levels modified the relationship between these genotypes and blood pressure in obese adolescents.

Discussion

The significant associations observed between adiposity measures and blood pressure in our study are consistent with the established role of obesity in adolescent hypertension. BMI percentile and waist circumference were positively associated with blood pressure, whereas, WtHR was not (p=0.072). WtHR performs inconsistently in pediatric population because ongoing growth affects both waist circumference and height until at least 18 years old, though a cut-off of >0.6 remains a useful risk-screening threshold in obese adolescents.(24,25) The higher hypertension risk among males aligns with existing evidence. The stronger effect observed in our study may reflect greater statistical power from the larger sample. Plausible mechanisms include gender difference in body composition, hormonal factors, and lifestyle behaviours and the cardioprotective effects of estrogen in females.(26,27)

Table 4. Association of *LSS* and *NEDD4L* gene polymorphisms with hypertension in obese adolescents (wildtype *vs.* variant).

Variant	Genotype	n (%)			OR	95% CI	*p*-value	*p*-trend
		Hypertension	Normotension	Total				
LSS A>C	Wildtype	8 (44.4)	10 (55.6)	18 (100)	0.783	0.29–2.07	0.6210[a]	0.6220[b]
	Variant	93 (50.5)	91 (49.5)	184 (100)				
	Total	101 (50.0)	101 (50.0)	202 (100)				
LSS A>G	Wildtype	31 (41.9)	43 (58.1)	74 (100)	0.597	0.33–1.06	0.0800[a]	0.0800[b]
	Variant	70 (54.7)	58 (45.3)	128 (100)				
	Total	101 (50.0)	101 (50.0)	202 (100)				
NEDD4L	Wildtype	67 (47.2)	75 (52.8)	142 (100)	0.683	0.37–1.25	0.2180[a]	0.2190[b]
	Variant	34 (56.7)	26 (43.3)	60 (100)				
	Total	101 (50.0)	101 (50.0)	202 (100)				

[a]Tested with Chi-square test. [b]Tested with Cochran–Armitage trend test.

Table 5. Minor allele frequency and Hardy–Weinberg Equilibrium test for *LSS* and *NEDD4L* gene polymorphisms by hypertension status.

Variant	Group	Minor Allele	n	MAF	HWE *p*-value
LSS A>C	Hypertension	A	67	0.332	0.1480
	Normotension	A	81	0.401	**0.0090**
LSS A>G	Hypertension	G	83	0.411	0.0900
	Normotension	G	69	0.342	0.7200
NEDD4L	Hypertension	A	39	0.193	0.4300
	Normotension	A	33	0.163	**0.0017**

MAF: Minor Allele Frequency; HWE: Hardy–Weinberg Equilibrium. Bold indicates significant deviation from equilibrium ($p<0.05$).

Our finding of no significant association between the urinary Na/K ratio and hypertension echoes a prior study in obese children, in which the ratio was numerically higher in the hypertensive group but did not reach significance.(28) The present study extends this observation to an Indonesian adolescent population with larger sample. Several factors may explain the lack of significant association: spot urine samples introduce inaccurate measurement relative to 24-hour collection (9,29), the heterogeneity in degree of obesity, metabolic profile, and dietary patterns. The pathophysiology of hypertension in obese adolescents may be dominated by obesity-related mechanisms rather than dietary electrolyte balance.(8)

This contrasts with previously published adult data such as the International Study of Salt and Blood Pressure (INTERSALT), which showed a robust sodium-blood pressure relationship (30), suggesting the pathophysiology may differ between adults and adolescents, or between general and obese populations. Adolescent studies are similarly mixed: the Wakayama Study found a significant Na/K urine-blood pressure association, but in broader, non-obese population.(11) Evidence that SNPs can modify the electrolyte-blood pressure relationship (31) and that population-specific genetic background influences blood pressure susceptibility (32) suggests gene–environment interactions may partly explain these inconsistent findings.

Table 6. Genotype dose-response trend in hypertension prevalence.

Variant	Genotype	Variant Allele Count	Hypertension (n)	Normotension (n)	% Hypertension within Genotype
LSS A>C	AA	0	8	10	44.4%
	AC	1	51	61	45.5%
	CC	2	42	30	58.3%
Cochran-Armitage trend test: *p*-trend = 0.1080					
LSS A>G	AA	0	31	43	41.9%
	AG	1	57	47	54.8%
	GG	2	13	11	54.2%
Cochran-Armitage trend test: *p*-trend = 0.1310					
NEDD4L	GG	0	67	75	47.2%
	GA	1	29	19	60.4%
	AA	2	5	7	41.7%
Cochran-Armitage trend test: *p*-trend = 0.4750					

Genotypes are ordered by increasing number of minor/variant alleles (variant allele count 0 = homozygous wildtype, 1 = heterozygous, 2 = homozygous variant); minor allele is C for *LSS* rs2254524, G for *LSS* rs2254522, and A for *NEDD4L* rs4149601. The % Hypertension within genotype = n (Hypertension) / [n (Hypertension) + n (Normotension)] for that genotype. The Cochran-Armitage trend test evaluates whether hypertension prevalence changes monotonically with allele dose; none of the three variants showed a statistically significant trend (all *p*-trend>0.05), consistent with the genotype-level and wildtype *vs.* variant comparisons.

Table 7. Results of the interaction test between urinary sodium and potassium levels and genotype on blood pressure in obese adolescents.

Interaction Variable	B	*p*-value	OR	95% CI
***LSS* Gene A>C × Sodium**				
Variant	0.002	0.4000	1.002	0.998-1.006
Wildtype	-0.005	0.2430	0.995	0.987-1.003
***LSS* Gene A>C × Potassium**				
Variant	0.002	0.7070	1.002	0.991-1.014
Wildtype	-0.012	0.2100	0.988	0.970-1.007
***LSS* Gene A>G × Sodium**				
Variant	0.002	0.4250	1.002	0.997-1.007
Wildtype	-0.001	0.7190	0.999	0.996-1.003
***LSS* Gene A>G × Potassium**				
Variant	0.010	0.1920	1.010	0.995-1.025
Wildtype	-0.007	0.1170	0.993	0.985-1.002
***NEDD4L* Gene × Sodium**				
Variant	0.000	0.9750	1.000	0.992-1.008
Wildtype	0.001	0.6290	1.001	0.998-1.003
***NEDD4L* Gene × Potassium**				
Variant	-0.009	0.3960	0.991	0.969-1.012
Wildtype	0.001	0.7240	1.001	0.995-1.007

Variant/Wildtype rows show the interaction coefficient (B), Wald *p*-value, odds ratio (OR), and 95% confidence interval for genotype × urinary sodium or genotype × urinary potassium interaction terms from unadjusted logistic regression models. Covariates such as sex and adiposity were excluded from these specific interaction models to evaluate the crude relationships. All 95% confident intervals (CIs) include 1.

The CC genotype of the *LSS* rs2254524 polymorphism appears more common among hypertensive individuals, suggesting a potential recessive pattern, though this association was non-significant and limited by small sample size. This contrasts with larger studies reporting significant associations between this variant and blood pressure, including the Hypertension in High School Students: Genetic and Environmental Factors (HYGEF) study involving 2,638 Italian adolescents showed that A-allele carriers had notably higher systolic and diastolic blood pressure.(33) Another study, in 828 obese children aged 6–17 years, also reported that A-allele carriers higher risk of stage I and II hypertension.(34) This discrepancy may reflect our limited power relative to those cohorts, differing hypertension diagnostic criteria (HYGEF used the percentile-based 2016 ESH guideline), and differences in ethnic composition affecting allele frequencies.(33,34) Mechanistically, the rs2254524 missense variant (Val642Leu) elevates *LSS* enzymatic activity and EO levels, modulating renal sodium handling. In adults, the *LSS* rs2254524 variant in AA genotype has been shown to be linked to salt sensitivity via EO regulation. It also diminishes the response to a low-salt diet, reducing treatment effectiveness, especially in individuals with AC and CC variants. It effect may emerge only after prolonged high-salt exposure, which adolescents may not have experienced yet and may be masked by the dominant influenced of adiposity and male sex.(13)

The additional *LSS* variant rs2254522 is a synonymous, benign change (per dbSNP/ClinVar CAT→CAC, no amino acid change at histidine ~650). It does not alter the protein sequence, consistent with our null finding. Effects via mRNA splicing or is in linkage disequilibrium with other functional *LSS* variants cannot be excluded without haplotype analysis.

NEDD4L rs4149601 polymorphism shows no significant association. The A allele favors the production of a C2-domain-deleted isoform, which has stronger ENaC binding and results in greater ENaC downregulation. The GG genotype has been linked to higher salt sensitivity and lower plasma renin.(35) Larger cohorts have reported blood pressure and cardiovascular effects tied to this variant, often modified by diuretic response.(36,37) The null finding here may reflect that *NEDD4L*'s effect on blood pressure is most evident in the context of sodium intake or diuretic response,

neither assessed here, as well as our focus on a single variant without haplotype data, and a distinct hypertension pathophysiology in obese adolescents.

A significant deviation from Hardy–Weinberg equilibrium was observed among normotensive participants for both *LSS* rs2254524 and *NEDD4L* rs4149601, but not in the hypertensive group. To rule out a technical explanation, two assessors independently re-read every chromatogram for these variants. Their calls agreed completely with each other and with the original genotyping (100% call rate and no missing data. Critically, the two deviations ran in opposite directions, rs2254524 carried an excess of heterozygotes whereas rs4149601 showed a deficit. Because a systematic genotyping artifact would be expected to bias consistently in one direction, this opposing pattern argues against a shared technical cause and points instead to chance across the multiple Hardy–Weinberg equilibrium tests performed, or to selection at these loci within the normotensive group. The heterozygotes excess for rs2254524 further argues directly against allele dropout and re-inspection at rs4149601 found no secondary peaks.

This study contributes data on genetic and environmental determinants of hypertension in obese Indonesian adolescents, a population that remains underrepresented in existing research. Neither the *LSS* and *NEDD4L* polymorphisms nor the urinary Na/K ratio was significantly associated with hypertension, suggesting that factors other than dietary sodium-potassium balance may dominate hypertension risk among in this population. Limitations of the study include the cross-sectional design and equal group sizes which preclude causal inference and reduce precision of prevalence estimates, unassessed pubertal status, reliance on spot Na/K ratio subject to misclassification, which tends to bias correlation estimates toward the null formal control for population stratification, though the predominantly Minangkabau makes substantial stratification unlikely. As an exploratory study, no multiplicity corrections were applied; however, since all primary *p*-values exceeded 0.05, such a penalty would not alter the conclusion. Larger, long-term studies in diverse pediatric populations are needed to see if genetic effects of *LSS* rs2254524, *LSS* rs2254522, and *NEDD4L* rs4149601 variant might also emerge later in adulthood.

Conclusion

Genetic variants of *LSS* rs2254524, *LSS* rs2254522, and *NEDD4L* rs4149601 are showing trends of hypertension among obese adolescents in this study. Greater adiposity and male gender, by contrast, were significantly associated with hypertension and remained the dominant correlates of blood pressure. This might be because in early adolescence, the strong neurohormonal and hemodynamic effects of severe adiposity may mask any subtle, long-term influences of these salt-handling gene variants.

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Authors Contribution

FA and AA contributed to the study's conceptualization, data collection, and investigation. FA, RM, and NIL developed the study methodology and statistical analyses. FA and RM contributed to drafting the manuscript. AA and NIL provided critical review and editorial revisions. All authors read and approved the final manuscript.

Ethical Statement

The protocol of this study have been approved by Ethical Committee of Faculty of Medicine, Universitas Andalas (Approval No. 274/UN.16.2/KEP-FK/2026). All subjects and their parents provided written informed consent and assent.

Conflict of Interest

The authors have no conflict of interests to disclose.

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