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GHSR gene polymorphism associated with lower weight-for-age Z-Score in children

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SUMMARY

Malnutrition remains a major public health issue, with ongoing efforts to identify effective strategies for its prevention. However, the genetic factors influencing child growth, particularly the role of the growth hormone secretagogue receptor (GHSR) gene, have not been sufficiently explored. This study aimed to investigate the correlation between GHSR gene polymorphisms and nutritional status in children from West Java, Indonesia. A cross-sectional study was conducted with 110 children aged 12–59 months. Weight and height measurements were taken to determine weight-for-age Z-scores (WAZ) and weight-for-height (WHZ), and dietary intake was assessed through a 24-hour food recall. Blood samples were collected for plasma extraction, and SNP analysis of the GHSR gene was performed using RT-PCR. The study revealed a high prevalence of underweight among the children, with 41.8% classified as underweight and 29.1% classified as wasted. A significant association was found between the TT genotype of the GHSR G57G polymorphism and lower WAZ compared to the CC and CT genotypes. This suggests that GHSR gene polymorphisms may play a role in malnutrition, specifically in influencing weight development. These findings highlight the potential influence of GHSR gene polymorphisms on nutritional status and child growth. Further research is needed to explore genetic factors contributing to malnutrition and to develop targeted interventions for improving child health outcomes.

KEYWORDS

GHSR gene, Malnutrition, SNPs, Under-Five Children, Underweight.

INTRODUCTION

Malnutrition remains a critical public health issue worldwide, affecting millions of children and posing long-term challenges for global development[1]. It arises from an imbalance between nutritional intake and the body's needs, leading to adverse health outcomes[2]. According to the World Health Organization (WHO), in 2022, approximately 149 million children under the age of five were stunted, 45 million were wasted, and 37 million were overweight. Alarmingly, nearly half of all deaths among children under five are linked to undernutrition[3]. Beyond its immediate health impacts, malnutrition also undermines social and economic progress, perpetuating cycles of poverty and poor health[4].

Research has identified malnutrition as a multifactorial condition influenced by a combination of direct and indirect factors[5]. Direct causes include inadequate nutritional intake and disease, while sensitive factors such as household income, feeding practices, hygiene and sanitation, healthcare access, and political, cultural, and social contexts significantly contribute to its prevalence[6]. Despite efforts to address these factors, malnutrition rates remain alarmingly high, suggesting that existing strategies are insufficient. This highlights the need to investigate other potential contributors, including genetic factors, which have been less explored but may play a critical role in growth outcomes.

Genetic factors, particularly the growth hormone secretagogue receptor (GHSR) gene, have recently garnered attention for its possible involvement in malnutrition[7,8]. Located on chromosome 3q26.31, the GHSR gene encodes a G-protein-

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coupled receptor that binds ghrelin, a hormone that stimulates appetite and regulates energy balance. This receptor plays a crucial role in the hypothalamic regulation of food intake, energy homeostasis, and growth hormone release, functions that are fundamental to normal growth and development[9,10]. Variations in this gene can disrupt its function, potentially leading to altered growth patterns. Previous studies have associated GHSR gene polymorphisms with body mass index (BMI)[11] and shorter stature[12]. However, despite growing interest in this area, further research is needed due to the limited number of populations and regions studied. This study aims to investigate the association between GHSR gene polymorphisms and nutritional status in children under five years old in West Java, Indonesia. The WAZ and WHZ is widely regarded as a reliable measure of nutritional status, offering a sensitive indicator of current nutritional intake and overall health. By exploring this genetic factor, the study seeks to contribute to the understanding of malnutrition's complex etiology and inform more targeted strategies for its prevention and management.

METHODS

Design and Subjects

This study employed a cross-sectional study involving a total of 110 under-five children living in two rural villages, Purwakarta Regency, West Java, Indonesia. Inclusion criteria were overall health, aged between 12-59 months, had no congenital diseases, and had no history of asphyxia. A minimum sample was 105 that calculated using Online Sample Size Estimator (OSSE) for genetic studies, with a power of 80% and a significance level of 5% (http://osse.bii.a-star.edu.sg/calculation1.php).

Data Collection

Nutritional Status

For children under two years of age, length was measured in a recumbent position. The child's head was positioned against the top of the measuring board, with eyes facing upward, legs fully extended, and feet perpendicular to the board. For children aged two years and older, height was measured in a standing position. The heels were kept together, and the back of the head, shoulders, buttocks, and heels were aligned with the vertical surface of a stadiometer. The head was positioned in the Frankfort horizontal plane. Length and height were measured using a multifunction stadiometer (Seca model 213, Seca GmbH & Co. KG, Germany). For children under two years, weight was measured in a recumbent position using a digital scale (SECA model 334, SECA Corp., Germany). For children aged two years and older, weight was measured in a standing position. All measurements were taken with children wearing minimal clothing. The procedures followed standard protocols and were conducted by trained personnel[13].

Dietary Intake

Dietary intake was evaluated through a 24-hour food recall conducted by trained nutritionists. Mothers were asked to report all foods and beverages their children had consumed in the past 24 hours, including details on food types, portion sizes, and preparation methods. This data was then used to calculate the intake of energy and nutrients (protein, carbohydrates, and fats) using Nutrisurvey software.

Blood Collection and GHSR Polymorphism Analysis

Approximately 5 ml of blood was aseptically collected from the antecubital vein for the purpose of genetic analysis of the GHSR gene. The blood was centrifuged at 2000 *g* for 15 minutes to separate the plasma, which was then transferred into sterile tubes and stored at -20°C until further analysis. DNA was extracted using a Genomic DNA mini kit (Geneaid, China) following the manufacturer's protocol. The concentration of DNA was measured using a Nanodrop EPOCH2 spectrophotometer (BioTek, USA), and the quality of the DNA was assessed by electrophoresis on a 1% agarose gel at 70V for 45 minutes, with visualization under UV light. Specific primers targeting the GHSR gene regions with known polymorphisms (including ΔQ36, G57G, P108L, L118L, R159R, C173R, D246A, and A277P) were designed based on the sequences reported by Inoue et al.[14] and synthesized by Integrated DNA Technologies (IDT, USA). The PCR was conducted in a 50 µl reaction mixture consisting of 25 µl MyTaq™ master mix, 4 µl DNA template, 2.5 µl of each primer (10 µM), and nuclease-free water to complete the volume. The PCR amplification protocol included an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds. This was followed by a final extension step at 72°C for 2 minutes. Amplification specificity was verified by including negative controls without DNA templates. PCR products were separated on a 1.5% agarose gel and visualized under ultraviolet light. Amplicons exhibiting a single, well-defined band of the expected size were selected for Sanger sequencing. Sequence data were analyzed using SnapGene Viewer and BioEdit software to identify GHSR gene polymorphisms.

Data Analysis

The WAZ and WHZ were computed using the WHO Anthro software. Prior to statistical analysis, the data were categorized according to standard WHO growth references. WAZ classifications included underweight (< –2 SD), normal (–2 SD to 2 SD), and overweight (> 2 SD). Similarly, WHZ was categorized as wasted (< –2 SD), normal (–2 SD to 2 SD), and overweight (> 2 SD). GHSR gene polymorphisms were grouped as G52G (CC, CT, and TT genotypes) and R159R (GG, GA, and AA genotypes). Age was divided

into two groups: 12–24 months and 25–59 months. Univariate analysis was performed to describe the frequency and percentage of each variable. Differences in nutritional status by age and gender were assessed using independent t-tests. Group comparisons for WAZ based on GHSR polymorphisms and nutrient intake were analysed using one-way ANOVA, followed by Duncan's post hoc test. Additionally, the distribution of genotypes was tested for Hardy-Weinberg equilibrium. A p-value of < 0.05 was considered statistically significant.

RESULTS

Subjects' Characteristics

A total of 110 children were included in this study, comprising 40.9% males and 59.1% females. The majority of participants (80.0%) were in the 2 to 5 years age group. Based on the WAZ, 41.8% of the children were classified as underweight (WAZ < –2 SD). Furthermore, based on the WHZ, approximately one-third of the children were categorized as wasted (WHZ < –2 SD). Detailed characteristics of the study subjects are presented in Table 1.

Further analysis was conducted to examine differences in nutritional status based on gender and age (Table 2). No statistically significant differences were found in WAZ or WHZ between male and female children. The mean WAZ for males was −1.76 ± 1.19, compared to −1.58 ± 1.31 for females ($p = 0.481$). Similarly, the WHZ were −0.88 ± 1.73 for males and −0.85 ± 1.19 for females ($p = 0.926$), indicating no significant gender-based differences in nutritional status. When analyzed by age group, children aged 13–23 months had a higher mean WAZ (−1.29 ± 1.54) than those aged 24–59 months (−1.75 ± 1.17), though the difference was not statistically significant ($p = 0.131$). A similar pattern was observed in WHZ, with younger children showing a more favorable nutritional status (−0.48 ± 1.96) compared to older children (−0.96 ± 1.26), but again without statistical significance ($p = 0.155$).

Table 1. Sociodemographic and Nutritional Status Characteristics of the Children

Characteristics	n = 110	%
Gender		
Male	45	40.9
Female	65	59.1
Age		
13 – 23 months	22	20.0
24 – 59 months	88	80.0
WAZ		
Underweight (<-2 SD)	46	41.8
Normal body weight (-2 SD – 2 SD)	64	58.2
Overweight (>2 SD)	0	0
WHZ		
Wasted (<-2 SD)	32	29.1
Normal (-2 SD – 2 SD)	68	61.8
Overweight (>2 SD)	10	9.1

WAZ = weight-for-age z-score; WHZ = weight-for-height z-score.

Table 2. Nutritional Status Differences based on Gender and Age

Characteristics	WAZ		WHZ	
	mean ± SD	p-value	mean ± SD	p-value
Gender				
Male	-1.76 ± 1.19	0.481	-0.88 ± 1.73	0.926
Female	-1.58 ± 1.31		-0.85 ± 1.19	
Age				
13 – 23 months	-1.29 ± 1.54	0.131	-0.48 ± 1.96	0.155
24 – 59 months	-1.75 ± 1.17		-0.96 ± 1.26	

WAZ = weight-for-age z-score; WHZ = weight-for-height z-score.
Statistical comparisons between groups were conducted using an independent t-test, with a significance threshold of $p < 0.05$.

Allele Frequency

Among the eight SNPs tested, only two (GHSR G57G and R159R) showed variation. The T allele was the more frequent variant for GHSR G57G (56%), whereas the G allele was predominant for GHSR R159R (63%). Both loci were tested for Hardy-Weinberg equilibrium. GHSR G57G showed no deviation (p = 0.822), while GHSR R159R was also not significantly deviated (p = 0.114), though closer to the threshold. Table 3 summarizes the allele frequencies and their corresponding Hardy-Weinberg equilibrium values.

Table 3. Allele Frequency and Hardy-Weinberg Equilibrium

Genes	Allele	Frequency	Hardy Weinberg Eq (p-value)
GHSR G57G	C	0.44	0.822
	T	0.56	
GHSR R159R	G	0.63	0.114
	A	0.37	

Dietary Intake, Nutritional Status and GHSR Polymorphisms

This study found a difference in WAZ between the genotypes of G57G, with the TT genotype showing lower WAZ compared to the wild-type CC genotype. However, no significant differences were observed for R159R. Furthermore, neither the G57G nor R159R genotypes were associated with energy and nutrient intake. These differences are presented in Table 4.

DISCUSSION

The present study identified a high prevalence of undernutrition among children, exceeding both global and national averages. Globally, in 2022, the prevalence of underweight and wasting among children under five was 15.4% and 6.8%, respectively[3]. Nationally, in 2023, the prevalence was reported at 15.9% for underweight and 8.5% for wasting[17]. The higher prevalence observed in this study may be attributed to the characteristics of the subjects, who were from rural villages often burdened by various social and economic challenges, including low household income, poor hygiene and sanitation, low maternal education, and limited access to food and healthcare facilities[18,19]. Additionally, the majority of the subjects were aged 24–59 months, a demographic where previous studies have shown a higher prevalence of undernutrition, particularly in children older than 24 months[20]. The present results also showed that subjects in this group had lower WAZ and WHZ scores compared to those in the 13–23-month age group. Furthermore, these factors collectively contribute to the increased risk of malnutrition among the study population.

This study identified two polymorphisms in the GHSR gene: G57G and R159R. Previous research has also reported SNPs in GHSR (rs292216 and rs509035) in obese female adolescents in Indonesia[21], though no association with dietary intake was found. However, subsequent studies have linked these polymorphisms to shorter stature[22]. In a 2021 Japanese study, SNPs in GHSR (G57G and Q35) were associated with the incidence of short stature[14]. Other studies have documented these genetic variations in various populations, including those in the Czech Republic[23], Egypt[24], and Brazil[25]. Additionally, SNPs in GHSR rs9819506-AA have been linked to lower body weight in individuals with glucose intolerance[26].

Table 4. Dietary Intake, Nutritional Status and GHSR Polymorphisms

Nutritional Status and Intake	G57G				R159R			
	CC (n = 23)	CT (n = 51)	TT (n = 36)	p-value	GG (49)	GA (n = 41)	AA (n = 20)	p-value
WHZ	-0.63 ± 1.21a	-0.81 ± 1.75a	-1.08 ± 0.99a	0.231	-0.64 ± 1.82a	-1.03 ± 1.02a	-1.06 ± 0.96a	0.284
WAZ	-1.17 ± 1.32b	-1.69 ± 1.25a,b	-1.90 ± 1.19a	0.089	-1.48 ± 1.35a	-1.86 ± 1.16a	-1.6 ± 1.22a	0.365
Energy (kcal)	1092.2 ±416.9	1111.1 ± 475.2	1118.1 ± 498.6	0.981	1084.5 ± 438.7	1083.9 ± 498.9	1228.5 ± 77.9	0.523
Protein (g)	33.6 ± 13.7	31.4 ± 12.6	38.2 ± 21.9	0.175	31.4 ± 13.0	36.3 ± 19.7	35.9 ±16.9	0.320
Carbohydrate (g)	146.9 ± 53.1	155.1 ± 87.6	148.1 ± 96.8	0.901	148.2 ± 81.3	145.4 ± 66.9	171.2 ± 120.2	0.519
Fat (g)	40.6 ± 24.4	41.7 ± 31.5	42.2 ± 26.9	0.978	42.2 ± 31.1	40.2 ± 27.2	43.4 ± 24.6	0.906
Fiber (g)	8.2 ± 19.8	5.4 ± 3.5	3.9 ± 2.9	0.251	6.8 ± 13.7	4.5 ± 3.1	4.5 ± 3.4	0.453

Despite finding no significant association between the SNPs in GHSR and dietary intake, the G57G variant showed a potential association with WAZ. Specifically, the TT genotype was associated with a lower WAZ compared to the CC and CT genotypes. The GHSR gene encodes a receptor that mediates the action of ghrelin, which is crucial for growth hormone release, appetite regulation, and energy balance[27]. Variations in this gene could alter receptor expression and, in turn, affect metabolic processes and growth. The G57G mutation is a synonymous mutation, which does not alter the amino acid sequence; however, it may still influence RNA function and gene expression by affecting mRNA folding, translation efficiency, and protein synthesis[28,29]. Previous studies have highlighted the clinical implications of synonymous mutations on metabolic health, including obesity and diabetes[30–32]. As this mutation occurs within an exon, it could disrupt the splicing process, leading to a non-functional GHSR protein, which would impair the regulation of growth hormone and energy homeostasis[33,34].

Although this study did not observe an association between SNPs and dietary intake, there are possible explanations. Similar studies employing food recall methods[21] also found no correlation between SNPs and dietary intake, yet a significant association with nutritional status was observed. We hypothesize that the effects of the SNPs may be mediated through alterations in the growth hormone axis or energy utilization mechanisms. These SNPs could potentially influence growth hormone secretion, leading to suboptimal IGF-I production. Reduced IGF-I activity could disrupt energy storage and contribute to suboptimal body composition development in children. However, there are some limitations to this study. The sample was derived from two urban villages, which may limit the generalizability of the findings. Additionally, this study utilized only a 24-hour food recall, which may not accurately represent the dietary intake of the subjects.

CONCLUSION

This study reveals a high prevalence of underweight among children in rural villages. Of the eight SNPs tested, two variants in the GHSR gene were identified: G57G and R159R. Although no significant association was found between these SNPs and dietary intake, the G57G variant showed a potential link to WAZ, with the TT genotype being associated with lower WAZ. These findings highlight the importance of considering genetic factors alongside environmental influences when assessing nutritional status. However, further research with a larger and more diverse sample is needed to confirm these results.

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