Gender Specificity and Local Socioeconomic Influence on Association of GHR fl/d3 Polymorphism With Growth and Metabolism in Children and Adolescents

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Objective: Growth hormone receptor (GHR) mediates most GH biological actions. This study is aimed to evaluate whether GHR fl/d3 polymorphism contributes to the inter-individual variability of growth and metabolism in healthy children and adolescents.

Methods: A total of 4,730 students aged 6-16 years from Yixing and Suqian City in China were included in this cross-sectional study. Height and body mass index (BMI) were transformed into the form of z-score corresponding to age and gender. Logistic regression was used to evaluate the associations of GHR fl/d3 polymorphism with height, BMI, metabolic traits, and hypertension by estimating the odds ratios (ORs) and 95% confidence intervals (CIs).

Results: GHR d3 allele was inversely associated with overweight, total cholesterol (TC) and triglyceride (TG) levels (OR [95% CI] for overweight: 0.754 [0.593-0.959], P = 0.021; OR [95% CI] for TC: 0.744 [0.614-0.902], P = 0.003; OR [95% CI] for TG: 0.812 [0.654-0.998], P = 0.047). GHR d3 allele was associated with decreased odds of pre-hypertension in boys (OR [95% CI]: 0.791 [0.645-0.971], P = 0.025), but associated with increased odds of pre-hypertension and hypertension in girls (ORs [95% CIs]: 1.379 [1.106-1.719], P = 0.004; OR [95% CI]: 1.240 [1.013-1.519], P = 0.037). Interaction of GHR fl/d3 polymorphism with gender contributed to increased odds of pre-hypertension and hypertension (interactive ORs [95% CIs]: 1.735 [1.214-2.481], P = 0.003; OR [95% CI]: 1.509 [1.092-2.086], P = 0.013). Stratification analysis showed that the correlation tendencies of GHR fl/d3 polymorphism and BMI with age were different between two cities with discrepant economic development levels.
INTRODUCTION

Growth hormone receptor (GHR), a member of the cytokine receptor family, mediates the majority of growth hormone (GH) biological actions (1), which plays a major role in the postnatal linear growth and metabolic activity during childhood and adolescence (2). In humans, there are two isoforms of recognized GHR transcripts, full-length GHR (GHR fl) and exon 3-deleted GHR (GHR d3) (3). It is of importance to investigate the genetic effects of GHR fl/d3 polymorphism on inter-individual variability of growth, metabolic traits, and blood pressure (BP) in general children and adolescent population.

Children growth and development is one of the important issues in public health (4). GH exerts multiple complex biological effects on carbohydrate and lipid metabolism such as decrease in insulin sensitivity, ensuing glucose intolerance, and protein anabolism (5). All these actions are mediated through its interaction with GHR. A previous study conducted in 48 Turner syndrome girls showed that, compared with GHR fl/fl and fl/d3 genotype, GHR d3 allele homozygote was associated with a unique GH responsiveness and had a lower body mass index (BMI) level (6). Another case-control study with 262 morbidly obese subjects reported that GHR d3 allele carriers had higher BMI than GHR fl allele individuals, but GHR fl/d3 polymorphism was not associated with any other components of metabolic syndrome (7). Another cohort study included 385 community healthy subjects followed from birth to adult life observed that GHR d3 allele was not associated with body height or weight in young adults (8), while these findings were contrary to Gao et al.’s findings performed in 715 children (9). Moreover, Turgut et al. found that systolic blood pressure (SBP) was significantly increased in GHR d3/d3 genotype carriers compared to GHR fl/d3 subjects in acromegalic patients (n = 35) (10). Park et al. also performed a genetic study in acromegalic patients (n = 30), but no significant association of GHR fl/d3 polymorphism and SBP or diastolic blood pressure (DBP) was found (11).

Thereby, results from the previous studies are contradictory, and no socioeconomic issue has been considered in those analyses. Our study was aimed to evaluate whether GHR fl/d3 polymorphism was associated with growth, metabolic traits, and hypertension risk with a relatively large healthy children and adolescent population.

MATERIALS AND METHODS

Study Population

This is a cross-section study that initially included a total of 6,160 subjects aged 6-16 years using a cluster sampling approach from Yixing and Suqian City in China (12, 13), which are, respectively, located in southern and northern Yangtze river (Supplementary Figure 1). Yixing City has a higher economic development level than Suqian City. One survey in Suqian City was conducted from October to November 2008 (n = 2,373), while another one in Yixing City was done in September 2014 (n = 3,787). All subjects were students from the local primary and junior high schools in a routine healthy examination program. The students aged under 6 years (n = 457), over 16 years (n = 40), and those with an incomplete physical examination record (n = 345) or refused to draw blood samples (n = 588) were excluded. Eventually, 4,730 children and adolescents were included in this study. The study protocols were approved by the Research Ethics Committee of Nanjing Medical University.

Anthropometric Measurements

Anthropometric data including body height, weight, and BP were measured by standard methods (14). Body height was measured to the nearest 0.1 cm using a standard column stadiometer placed on a level ground. The students stood upright on bare feet, with heels together, and buttocks and back touching the meter rule. Body weight was measured to the nearest 0.1 kg using an electronic weightier, and the children were weighed wearing minimum clothing and without shoes. Both height and weight were measured twice. BMI value was calculated as weight (kg)/height squared (m²). Height and BMI were transformed to a z-score corresponding to age and gender (15), and normalized according to a nationwide study of height and weight standardized growth charts (16). All participants were classified into “height z-score < −1, −1 ≤ height z-score < 1 and height z-score ≥ 1” groups, according to the World Health Organization (WHO) reference of height for different age and gender groups (17). In addition, subjects were divided into low body weight, overweight, and obesity groups based on the cut-off points of the 3rd, 85th, and 95th centiles of BMI for age and gender (18, 19).

BP measurements were performed twice after resting for at least 5 min under a quiet position using the electronic sphygmomanometers (OMROM, HEM-7207, Dalian, China) according to a standard protocol (20). If the difference between these two measurements of either SBP or DBP was over 8 mmHg, an additional measurement was needed. Finally, the average values of two or three measurements of SBP and DBP were calculated for analysis. Likewise, BP value was transformed into BP z-score corresponding to age, gender, and height (21).

According to the reference values of "The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents" (22), the subjects were classified into normal BP, pre-hypertension, and hypertension groups. The

Conclusion: GHR fl/d3 polymorphism is associated with growth, metabolism, and hypertension in children and adolescents with the gender specificity, and the genetic effect of GHR fl/d3 may be modified by the local socioeconomic levels.

Keywords: gender specificity, GHR fl/d3 polymorphism, growth hormone receptor, metabolism, socioeconomic levels
normal BP group was defined as both SBP and DBP <90th percentile; average SBP and/or DBP at least 90th, but <95th, and/or SBP ≥ 120 or DBP ≥ 80 mmHg regardless of age, gender, and height was divided into the pre-hypertension group; the hypertension group was identified as the average SBP and/or DBP persistently above the 95th percentile, and/or SBP ≥ 140 or DBP ≥ 90 mmHg regardless of age, gender, and height.

**Biochemical Indices**

Non-fasting peripheral venous blood was retained, and serum TC, TG, and GLU concentrations were measured by an automated biochemical profiling (Mindray BS-800, Shenzhen, China). Because there were no specific cut-off values to identify the relative high levels of TC, TG, and GLU in children and adolescents, we calculated the value of the 90th percentile of TC, TG, and GLU to classify the students into normal group and relative high-level group in the association analyses.

**GHR fl/d3 Genotyping**

DNA was extracted from the leukocyte of peripheral blood by using standard phenol-chloroform method (23). The polymerase chain reaction (PCR) method was used for genotyping GHR fl/d3 polymorphism in a 384-well plate. PCR was conducted in 10 μl of a reaction mixture containing about 10 ng of DNA sample, 1 μl reaction buffer, 1 μl MgCl2, 0.15 μl of platinum Taq DNA polymerase, and 100 pmol each of primer. This assay was carried out with primers G1, G2, and G3 (GenBank association no. AF155912) as follows (3): initial step of denaturation of 5 min at 95°C followed by 39 cycles consisting of 30 s at 95°C, 30 s at 64.8°C, 1 min 30 s at 72°C, followed by an extension period of 72°C for 10 min. Application of DNA fragments was analyzed by electrophoresis on a 1.5% agarose gel and visualized by ethidium bromide staining under ultraviolet (UV) light. The polymorphism detected by PCR was evident as a 592 bp fragment in the presence of the deletion (d3/d3) and a 935 bp product in the presence of the full-length fragment (fl/fl) of exon 3 in the GHR gene. Each sample was genotyped as GHR d3/d3, fl/d3, or fl/fl.

**Statistical Analysis**

Continuous variables were expressed as mean ± standard deviation (SD) or as median (interquartile range). Independent sample t-test and analysis of variance (ANOVA) were used to analyze differences in continuous variables with normal distribution. Mann–Whitney U-test and Kruskal–Wallis test were used to analyze differences in continuous variables with non-Gaussian distribution. A chi-square test \((\chi^2)\) was used for comparison of nominal variables between groups.

In the primary analyses, multivariate logistic regression model was used to estimate the associations of GHR fl/d3 polymorphism with height z-score levels (reference group: \(-1 \leq \text{height } z\)-score < 1 group), obesity (reference group: normal weight group), and hypertension (reference group: normotensive group), while the binary logistic regression model was applied in the analysis of GHR fl/d3 polymorphism with metabolic traits using additive (fl/fl vs. fl/d3 vs. d3/d3), dominant (fl/fl + fl/d3 vs. d3/d3), and recessive (fl/fl vs. fl/d3 + d3/d3) models. Odds ratio (OR) and 95% confidence interval (CI) were calculated to quantify these associations. In the secondary analyses, we explored the modification effect of socioeconomic levels on the associations of GHR fl/d3 polymorphism with childhood height, BMI, metabolic traits, and hypertension through a stratification analysis. To evaluate the relationship of GHR fl/d3 polymorphism and BMI z-score among the subjects in different age groups, the consecutive tendencies of predicted BMI z-score by age stratified by gender and region were separately depicted by using the quadratic prediction plot. Besides, to explore the potential interaction between GHR fl/d3 polymorphism and gender on hypertension risk, Breslow-Day test for heterogeneity was performed and interaction figures were depicted using R-package “descTools” and “meta.” All the statistical analysis above was performed using Stata version 16.0 (Stata Corp LLC, College Station, TX) and R package (version 3.6.1). Two-tailed P-value < 0.05 was defined to be statistically significant.

**RESULTS**

**Characteristics of the Participants**

Demographic and clinical characteristics according to the genotypes of the GHR exon 3 polymorphisms were summarized in Table 1. A total of 4,730 students (2,465 boys and 2,265 girls) were included in the current study, with an average (SD) age of 10.83 (2.96) years. Totally, the numbers of GHR fl/fl, fl/d3, and d3/d3 genotype carriers were 3,287 (69.5%), 1,267 (26.8%), and 176 (3.7%), respectively. The percentage of GHR fl/fl, fl/d3, and d3/d3 carriers was significantly different among boys and girls with a P-value of 0.024. But no significant difference of age, height z-score, BMI z-score, SBP z-score, DBP z-score, TC, TG, and GLU was found among the three GHR genotypes.

**Associations of GHR fl/d3 Polymorphism With Height, BMI, and Hypertension**

No statistical association of GHR d3 polymorphism and height z-score was observed in total subjects. In boys, the GHR d3 allele was significantly associated with lower height z-score, and ORs (95% CIs) for the additive model and dominant model were 1.221 (1.009-1.478) and 1.285 (1.016-1.624) with P-values of 0.040 and 0.036, respectively (Table 2). In girls, an inverse association was observed between GHR d3 allele and height z-score, and ORs (95% CIs) for the additive model and dominant model were 0.773 (0.614-0.972) and 0.749 (0.577-0.972) with P-values of 0.028 and 0.030, respectively.

GHR d3 allele variation was associated with decreased odds of overweight in the entire population and ORs (95% CIs) for the additive model and dominant model were 0.754 (0.593-0.959) and 0.738 (0.561-0.971) with P-values of 0.021 and 0.030, respectively (Table 3). In boys, similar association of GHR d3 allele with overweight was observed (OR [95% CI] for the additive model was 0.717 [0.515-0.998], \(P = 0.049\)), while no significant association was found in girls.

No statistical associations of GHR d3 polymorphism with pre-hypertension or hypertension were observed in total subjects. Boys with GHR d3 allele were associated with decreased odds of pre-hypertension, and ORs (95% CIs) for the additive model
and dominant model were 0.791 (0.645-0.971) and 0.777 (0.609-0.990) with \( P \)-values of 0.025 and 0.042, respectively (Table 4). In girls, \( GHR \) d3 allele was associated with increased odds of pre-hypertension and hypertension, and ORs (95% CIs) for the additive model were 1.379 (1.106-1.719) and 1.240 (1.013-1.519) with \( P \)-values of 0.004 and 0.037, respectively.

**Table 1**: Demographic and clinical characteristics for the participants.

| Variable                      | Group       | Total            | \( \text{fl/fl} \) | \( \text{fl/d3} \) | \( \text{d3/d3} \) | \( P \)-value |
|-------------------------------|-------------|------------------|------------------|------------------|------------------|-------------|
| Gender (n/%)                  |             |                  |                  |                  |                  |             |
| Boys                          | 2,465 (52.1)| 3,287 (69.5)     | 1,267 (26.8)     | 176 (3.7)        |                  | 0.024       |
| Girls                         | 2,265 (47.9)| 1,691 (68.6)     | 665 (27.0)       | 109 (4.4)        |                  |             |
| Age (year)                    |             | 10.83 ± 2.96     | 10.84 ± 2.95     | 10.83 ± 3.01     | 10.70 ± 3.03     | 0.836       |
| Height z-score                |             | 0.11 (-0.72,0.86)| 0.12 (-0.73,0.84)| 0.12 (-0.7,0.89)| 0.14 (-0.63,0.93)| 0.858       |
| BMI z-score                   |             | -0.68 (-0.19,0.43)| -0.18 (-0.66,0.48)| -0.19 (-0.68,0.45)| -0.21 (-0.69,0.45)| 0.506       |
| SBP z-score                   |             | 0.52 (-0.37,1.31)| 0.52 (-0.37,1.31)| 0.49 (-0.42,1.29)| 0.68 (-0.41,1.36)| 0.386       |
| DBP z-score                   |             | 0.26 (-0.14,0.75)| 0.27 (-0.14,0.76)| 0.25 (-0.18,0.72)| 0.31 (-0.03,0.82)| 0.246       |
| TC (mmol/L)                   |             | 3.83 ± 0.57      | 3.76 ± 0.76      | 3.74 ± 0.74      | 3.66 ± 0.65      | 0.153       |
| TG (mmol/L)                   |             | 1.24 ± 0.74      | 1.07 ± 0.67      | 1.07 ± 0.63      | 1.01 ± 0.66      | 0.511       |
| GLU (mmol/L)                  |             | 4.51 ± 1.17      | 4.45 ± 1.10      | 4.43 ± 1.04      | 4.43 ± 1.08      | 0.920       |

Continuous variables with normal distribution were expressed as means ± standard deviation. Continuous variables with non-Gaussian distribution were expressed as median (interquartile range). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; GLU, glucose.

**Table 2**: Association analysis of \( GHR \) fl/d3 polymorphism with height.

| Subject | Height z-score group | WT/HT/MT | Additive model | Dominant model | Recessive model |
|---------|----------------------|----------|----------------|----------------|----------------|
|         |                      |          | \( OR \) (95% CI) | \( P \)-value | \( OR \) (95% CI) | \( P \)-value | \( OR \) (95% CI) | \( P \)-value |
| Total subjects |                  |          |                  |                |                |                |                |
|         | -1~1                 | 2.117/810/109 | Reference          | Reference      | Reference      |                |                |
|         | < -1                 | 529/202/28  | 1.004 (0.869-1.162) | 0.957 | 1.002 (0.842-1.191) | 0.985 | 1.024 (0.671-1.563) | 0.912 |
|         | ≥1                   | 641/255/39  | 1.059 (0.928-1.209) | 0.396 | 1.057 (0.902-1.238) | 0.496 | 1.164 (0.801-1.690) | 0.426 |
| Boys    | -1~1                 | 1.049/385/60 | Reference          | Reference      | Reference      |                |                |
|         | < -1                 | 255/119/20  | 1.221 (1.009-1.478) | 0.040 | 1.285 (1.016-1.624) | 0.036 | 1.272 (0.757-2.137) | 0.364 |
|         | ≥1                   | 387/161/29  | 1.138 (0.962-1.348) | 0.132 | 1.157 (0.941-1.422) | 0.166 | 1.259 (0.799-1.982) | 0.321 |
| Girls   | -1~1                 | 1.068/425/49 | Reference          | Reference      | Reference      |                |                |
|         | < -1                 | 274/83/8    | 0.773 (0.614-0.972) | 0.028 | 0.749 (0.577-0.972) | 0.030 | 0.680 (0.319-1.449) | 0.318 |
|         | ≥1                   | 254/94/10   | 0.929 (0.746-1.156) | 0.508 | 0.923 (0.717-1.188) | 0.534 | 0.872 (0.437-1.739) | 0.697 |

WT, fl/fl genotype; HT, fl/d3 genotype; MT, d3/d3 genotype; OR, odds ratio; CI, confidence interval.

**Associations of \( GHR \) fl/d3 Polymorphism With Metabolic Traits**

In total population, \( GHR \) d3 allele was inversely associated with higher TC level, and ORs (95% CIs) of additive, dominant, and recessive models were 0.744 (0.614-0.902), 0.757 (0.608-0.942), and 0.372 (0.173-0.799) with \( P \)-values of 0.003, 0.013, and 0.011, respectively (Table 5). The significant association between \( GHR \) d3 allele and higher TC level was further observed in the subgroup of boys and girls, and ORs (95% CIs) of additive model were 0.742 (0.565-0.973) and 0.747 (0.568-0.982) with \( P \)-values of 0.031 and 0.037, respectively. \( GHR \) d3 allele was inversely associated with higher TG level and OR (95% CI) was 0.812 (0.654-0.998) with a \( P \)-value of 0.047.

**Stratification Analysis**

Stratification analysis of \( GHR \) fl/d3 polymorphism with height, BMI, hypertension, and metabolic traits by region was performed (Supplementary Tables 1–4). \( GHR \) d3 variation was associated with increased odds of low BMI levels in Suqian boys and OR (95% CI) of additive model was 1.323 (1.034-1.693) with \( P \)-value of 0.049. No significant

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associations between GHR d3 variation and height levels were observed among Yixing and Suqian students.

Compared with GHR fl/fl and fl/d3 genotypes, a unimodal shape of GHR d3/d3 on BMI z-score by age was visually observed in boys and girls (Supplementary Figure 2). This tendency of GHR d3/d3 on BMI was further displayed in Yixing and Suqian city (Supplementary Figure 3). After we stratified by region and gender, a similar effect of GHR fl/d3 and d3/d3 genotypes on BMI was observed in Yixing boys, while the effect of GHR d3/d3 on BMI was inversed in Yixing girls. For GHR fl/fl and fl/d3 carriers in Suqian, BMI z-score was decreased with the increase of age in boys, which was contrary to the d3/d3 genotype. In Suqian girls, the fluctuating range of GHR d3/d3 on BMI was obviously enlarged and its peak approximately appeared in the 10- or 11-year groups (Supplementary Figure 4). GHR d3 allele was associated with reduced odds of pre-hypertension and hypertension in Yixing boys (ORs [95% CIs] for the additive model and dominant model: 0.750 [0.585-0.962], \( P = 0.023 \); 0.757 [0.587-0.976], \( P = 0.031 \); Supplementary Table 3). Conversely, GHR d3 allele was associated with increased odds of pre-hypertension and hypertension in Yixing girls (ORs [95% CIs] for the additive model: 1.366 [1.055-1.770], \( P = 0.018 \); 1.273 [1.009-1.606], \( P = 0.042 \)). No significant associations were found between GHR fl/d3 polymorphism and hypertension in Suqian City.

### TABLE 3 | Association analysis of GHR fl/d3 polymorphism with BMI.

| Subject | BMI group | WT/HT/MT | Additive model | Dominant model | Recessive model |
|---------|-----------|----------|----------------|----------------|----------------|
|         |           |          | OR (95% CI) | \( P \)-value | OR (95% CI) | \( P \)-value | OR (95% CI) | \( P \)-value |
| Total subjects | Normal weight | 2,001/786/103 | Reference | 0.369 | 0.692 | 0.107 |
|         | Low weight | 826/223/56 | 1.057 (0.937-1.193) | 0.306 | 0.135 (0.943-1.836) | 0.107 |
|         | Overweight | 225/88/6 | 0.754 (0.593-0.959) | 0.021 | 0.553 (0.240-1.270) | 0.163 |
|         | Obesity | 235/90/11 | 0.965 (0.783-1.189) | 0.736 | 0.914 (0.485-1.720) | 0.779 |
| Boys | Normal weight | 1,071/418/65 | Reference | 0.953 | 0.774 | 0.580 |
|         | Low weight | 381/161/35 | 1.153 (0.978-1.360) | 0.090 | 1.486 (0.973-2.270) | 0.067 |
|         | Overweight | 118/37/2 | 0.717 (0.515-0.998) | 0.049 | 0.297 (0.072-1.225) | 0.093 |
|         | Obesity | 121/49/7 | 1.008 (0.764-1.33) | 0.953 | 0.948 (0.427-2.101) | 0.895 |
| Girls | Normal weight | 930/368/38 | Reference | 0.953 | 0.774 | 0.580 |
|         | Low weight | 455/162/21 | 0.971 (0.812-1.162) | 0.010 | 1.165 (0.678-2.003) | 0.580 |
|         | Overweight | 107/31/4 | 0.801 (0.565-1.136) | 0.213 | 0.976 (0.343-2.777) | 0.964 |
|         | Obesity | 114/41/4 | 0.914 (0.665-1.255) | 0.914 | 0.869 (0.306-2.468) | 0.792 |

**WT, fl/fl genotype; HT, fl/d3 genotype; MT, d3/d3 genotype; OR, odds ratio; CI, confidence interval.**

### TABLE 4 | Association analysis of GHR fl/d3 polymorphism with pre-hypertension and hypertension.

| Subject | Blood pressure group | WT/HT/MT | Additive model | Dominant model | Recessive model |
|---------|----------------------|----------|----------------|----------------|----------------|
|         |                      |          | OR (95% CI) | \( P \)-value | OR (95% CI) | \( P \)-value | OR (95% CI) | \( P \)-value |
| Total subjects | Normotensive | 2,166/846/109 | Reference | 0.819 | 0.793 | 0.285 |
|         | Pre-hypertension | 487/187/28 | 1.018 (0.877-1.181) | 0.067 | 1.133 (0.742-1.731) | 0.562 |
|         | Hypertension | 634/234/39 | 1.010 (0.883-1.156) | 0.081 | 1.226 (0.844-1.781) | 0.285 |
| Boys | Normotensive | 1,045/444/75 | 0.971 (0.812-1.162) | 0.749 | 1.165 (0.678-2.003) | 0.580 |
|         | Pre-hypertension | 287/99/12 | 0.971 (0.812-1.162) | 0.749 | 1.165 (0.678-2.003) | 0.580 |
|         | Hypertension | 359/122/22 | 0.971 (0.812-1.162) | 0.749 | 1.165 (0.678-2.003) | 0.580 |
| Girls | Normotensive | 1,121/402/34 | 0.971 (0.812-1.162) | 0.749 | 1.165 (0.678-2.003) | 0.580 |
|         | Pre-hypertension | 200/88/16 | 1.379 (1.106-1.719) | 0.004 | 2.456 (1.338-4.508) | 0.004 |
|         | Hypertension | 275/112/17 | 1.240 (1.013-1.519) | 0.037 | 1.942 (1.073-3.513) | 0.028 |

**WT, fl/fl genotype; HT, fl/d3 genotype; MT, d3/d3 genotype; OR, odds ratio; CI, confidence interval.**
Interaction Between GHR fl/d3 Polymorphism and Gender on Hypertension

Breslow-Day test showed significant interactive effects of GHR fl/d3 polymorphism and gender on pre-hypertension and hypertension risk existed, and the P-values of the homogeneity test were 0.002 and 0.013, respectively (Supplementary Figure 5). GHR fl/d3 dominant model had positive multiplicative interactions on pre-hypertension and hypertension (interactive ORs [95% CIs]: 1.735 [1.214-2.481], P = 0.003; 1.509 [1.092-2.086], P = 0.013; Supplementary Tables 5, 6).

DISCUSSION

In the current study, we investigated the associations of GHR fl/d3 polymorphism with height, BMI, hypertension, and metabolic traits in healthy children and adolescents with the largest sample size. We observed the multiplicative interaction of GHR fl/d3 polymorphism with gender contributing to increased odds of pre-hypertension and hypertension and found that GHR d3 allele was likely to have a protective effect against high TC level. Furthermore, genetic effect of this polymorphism on BMI was inconsistent in different age groups, which may be modified by the local socioeconomic levels.

Hypertension in children and adolescents has been recognized as a challenge (24), to which genetic polymorphism and their interaction with environmental factors may contribute (25). Lack of GHR signaling causes a reduction in SBP and plasma renin levels as well as an increase in aortic eNOS expression (26). Animal studies showed GHR knockout mice led to a 25% reduction in SBP compared with wild-type mice (26). In the current study, we assessed the associations of this polymorphism with hypertension status in general childhood population. GHR d3 allele is found to be associated with decreased odds of pre-hypertension and hypertension in boys, while increased odds of pre-hypertension and hypertension in girls was observed. Furthermore, interaction between GHR fl/d3 polymorphism and gender on pre-hypertension and hypertension was first illustrated, suggesting genetic effects of this polymorphism may have a gender specificity.

Socioeconomy is a conspicuous environmental factor that affects physical growth in children. Yixing and Suqian are two cities located in south and north of the Yangtze River, China. Traditionally and currently, the socioeconomic level in Yixing is significantly higher than that in Suqian (Supplementary Figure 1). These two cities are different not only in geography but also in social developing stages from history until now. We thus separately analyzed the associations of GHR fl/d3 polymorphism with growth and metabolic traits in children and adolescents from Yixing and Suqian Cities. Data demonstrated that the carriers with GHR d3 allele are
associated with low BMI levels in Suqian children. A similar trend of this association in Yixing children was observed but not statistically significant. These findings are consistent with the previous studies (9, 27). The association of GHR d3 allele with low BMI is speculated that biological function of this allele may increase lipolytic effect (28), which is helpful in keeping a relatively low body fat mass composition (29). We have further depicted the different GHR genotypes of BMI fluctuation among 6-16 years age groups in boys and girls. The large swing of BMI variation among GHR fl/d3 genotypes with age was displayed in Yixing and Suqian, indicating that genetic effects of GHR fl/d3 polymorphism on childhood BMI could be modified by the local socioeconomic levels.

A previous study has reported that GHR d3 allele had a 30% higher bioactivity than GHR fl allele (27). Data from the current study demonstrated that GHR d3/d3 on BMI had a larger fluctuation with age than fl/fl and fl/d3 genotypes. Together with the implication from the previous study (27), our data consistently suggest that GHR d3 allele may have a vital role on BMI in children and adolescents. A unimodal peak of GHR d3/d3 on BMI with age approximately appeared at 10 or 11 years group, indicating the relationship between GHR fl/d3 polymorphism and BMI is significantly different at puberty in boys and girls. Stratified by region and gender, compared with Suqian City, the effect of GHR d3/d3 genotype on BMI was relatively moderated in Yixing City. This finding may also support our postulate that socioeconomic modifies the genetic effect of GHR fl/d3 polymorphism on BMI. Furthermore, the direction of association of GHR d3 allele on height levels was opposite in boys and girls, further investigations to understand how puberty interacts with GH in the regulation of BMI and height could be taken into consideration.

Previous studies showed that no significant difference of lipids profile in GHR fl/d3 genotype carriers in children and adolescents was observed (7, 8). The relatively small sample sizes of those studies may be a likely reason for the null association. Whether GHR fl/d3 polymorphism associates with metabolic traits is still not well investigated. Our study presented GHR d3 allele may have a negative relationship with higher levels of TC and TG, suggesting that the GHR d3 allele may have a protective function against the abnormal lipid metabolism. One possible explanation is that the bioactivity of GHR regulated the carbohydrate and lipid metabolism via several downstream insulin signaling events (30).

Several limitations existed in this study. First, potential confounding factors, such as calorie intake and physical exercise, that may introduce bias by modifying the associations between GHR fl/d3 polymorphism, BMI, metabolic traits, and hypertension were not examined. Second, this population with Han nationality were from east China, the generalizability of our findings to other regions needs further validation. Third, in this study, the distribution of GHR fl/d3 polymorphism was deviated from Hardy-Weinberg equilibrium (HWE) in the total population. Nevertheless, we excluded the genotyping error by checking the raw data of the genotyping results among each batch, where no large deviation of GHR fl/d3 frequency among each batch was found. Thus, the interpretation of our findings could be convincing to some extent in the case of departure from HWE. Moreover, the exploratory nature of our study without a prespecified power calculation precludes us from drawing any confirmative conclusions.

In conclusion, the current study demonstrated that GHR fl/d3 polymorphism was associated with BMI, metabolism, and hypertension in children and adolescents, which may be modified by local socioeconomic levels.

**DATA AVAILABILITY STATEMENT**

The datasets generated for this study are available on request to the corresponding author.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by Research Ethics Committee of Nanjing Medical University. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

**AUTHOR CONTRIBUTIONS**

HG and CS proposed and designed the study. CL, XC, SY, CN, QZ, YY, YC, XZ, WZ, and QZ collected the data. CL, XC, XH, SY, and CS carried out all analyses. CL and XC wrote the draft. WY, CS, and HG edited the manuscript. All authors contributed to data interpretation, discussion, and revision of the article.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.546080/full#supplementary-material
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