Original Article
Association of SNPs in *GHSR* rs292216 and rs509035 on dietary intake in Indonesian obese female adolescents

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Abstract: Background: Obesity has been linked to high dietary intake and low physical activity. Studies showed that those factors were not only regulated by environment but also by genetic. However, the relationship is less been understood in obese children and adolescents. Objective: The objective of this study was to examine the role of SNPs in *GHSR* rs292216 and rs509035 on dietary intake in obese female adolescents. Methods: This is an observational study with cross sectional design. Respondents were obese female adolescents enrolled from obesity screening done in six junior high schools in Yogyakarta. Dietary intake was measured using 6 days 24 hours inconsecutive dietary recall. Genotyping of 2 SNPs from *GHSR* was done using FRLP-PCR. Results: There were 78 obese female adolescents joined this study. We found that no significant association between SNPs *GHSR* and dietary intake (*p* > 0.05). In addition, a SNP-SNP interaction analysis shown there is no difference between combination of *GHSR* rs292216 and rs509035 on dietary intake (*p* > 0.05). Conclusion: We concluded that SNPs on *GHSR* rs292216 and rs509035 were not related to dietary intake in Indonesian obese female adolescents. Further study is necessary to investigate the effect of those genes on dietary intake in the broader population.

Keywords: GHSR, dietary intake, obesity, adolescents, female

Introduction

World's population of obese children and adolescent has been markedly increasing over the last 2 decades [1, 2]. In Indonesia, it has recently been reported that the prevalence of overweight in adolescents increased drastically from 1.4% in 2007 to 7.3% in 2013 [3]. Eating habits such as snacking, binge eating and overeating as well as low physical activity have been associated with increasing risk of obesity [4-7]. There are evidences showing that eating pattern is not purely controlled by environmental factors but also genetically inherited [8].

GHSR [9] is a gene previously reported to be associated with dietary habit and have been reported associated with risk of obesity. The gene encodes growth hormone secretagogue receptor, an important receptor in signalling system of ghrelin hormone that influence appetite and eating habit [10]. Although some studies have supported the effect of *GHSR* gene polymorphism with obesity, there is a lack of information regarding the role of SNPs on dietary intake in obese female adolescents. Knowing the effect of SNP in *GHSR* is important because that is potential to be used as a predictor of dietary changes during lifestyle intervention in obese individuals. Therefore, in this study we aimed to gain knowledge on the role of single nucleotide polymorphisms (SNPs) of *GHSR* on dietary intake in obese female adolescents.

Methods

Subjects were selected based on obesity screening of 2120 female adolescents from six junior high schools in Yogyakarta. All obese girls

Table 1. Primers and restriction enzymes used for PCR-RFLP

Gene	Primers (forward)	Primers (reverse)	Restriction enzymes	Incubation temperature and time
GHSR rs292216	5'AATCCCAGTCCTTT GTATCACTCGGT3'	5'AGTTATGCCTCAA AAAATGTTTCCCG3'	BsrBI	Overnight (37°C)
GHSR rs509035	5'GAGGATGAAG GCAAAC3'	5'CCAGGAGAC TCGGATA3'	SacI	1 hour (37°C)

Table 2. Characteristics of subjects

Characteristics (n = 78)	Mean ± SD
Age (years)	13.7 ± 0.9
Body weight (kg)	70.5 ± 8.3
Height (cm)	154.0 ± 5.4
Body mass index (kg/m2)	29.7 ± 2.6
Dietary intake	
Energy (kcal)	1,141.0 ± 306.3
Protein (g)	40.0 ± 11.6
% Protein per energy	14.1 ± 2.3
Fat (g)	41.9 ± 13.9
% Fat per energy	32.8 ± 5.4
Carbohydrate (g)	151.2 ± 42.9
% Carbohdrate per energy	53.1 ± 6.1

Table 3. Allele frequency and Hardy-Weinberg Equilibrium

Genes	Alleles	Frequency	Hardy-Weinberg Eq (p value)
GHSR rs292216	T	0.54	0.82
	A	0.46	
GHSR rs509035	G	0.66	0.01
	A	0.34	

also analysed as a relative intake to total energy intake based on the amount of energy obtained from all those components.

DNA sample was extracted from peripheral blood (5 ml) using salting out methods. Genotyping of those six genes was done using PCR-RFLP with primers as shown in **Table 1**. The PCR conditions were 94°C for 3 min; followed by 35 cycles of 94°C for 30 second, 65°C for 30 second, 72°C for 30 second and 72°C for 5 minutes. After amplification, PCR products were incubated using enzymes that are shown in **Table 1**. The fragments were resolved on 3% agarose gel for electrophoresis.

Allele frequency, Hardy Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) were analysed. In order to analyse the relationship between genetic variations and dietary intake and physical activity, one way ANOVA was used. In addition, several analysis using dominant model, recessive model, over dominant model and log additive models were used for confirmation on analysis. A student t-test was used to compare dietary intake between 2 genotypes.

A SNP-SNP interaction analysis was done to evaluate the relationship between combination genotypes of *GHSR* rs292216 and rs509035 with dietary intake. In this analysis, each genotypes between SNPs were combined thus the phenotypes of each combination were compared. Combination of TT from GHSR rs292216 and GG from GHSR rs509035 were used as reference. There is two type of analysis done in this study 1) t-test to analyze the difference between energy, carbohydrate, fat and protein intake as well as ratio carbohydrate, fat and protein to total energy intake (data not shown); 2) fisher-exact to analyze the relationship between each haplotype and high fat intake. All analysis were done using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA.

were invited to join the research as participants with the informed consent signed by their parents. Obesity status was defined as body mass index (BMI) above 95th percentile from 2000 Growth Reference Standard (WHO-Centre for Disease Control). Anthropometric standard to define obesity has been published elsewhere (Muhammad, 2009). Ethical clearance was obtained from Ethical Commission of Medical and Health Research, Universitas Gadjah Mada, Yogyakarta.

Dietary intake and physical activity was obtained by interview. A six non-consecutive 24 hours dietary recall was used to measure dietary intake including total energy intake, protein, fat and carbohydrate as well as proportion of protein, fat and carbohydrate to total energy intake (%). Nutrisurvey was used as a tool to obtain data on nutrient intake from dietary recall. Data on protein, fat and carbohydrate

Table 4. Genetic variation and dietary intake

	GHSR rs292216							GHSR rs509035						
	A/A (n = 7)		A/T (n = 9)		T/T (n = 62)		p_{ANOVA}	A/A (n = 4)		A/G (n = 45)		G/G (n = 28)		p_{ANOVA}
	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	
Energy (kcal)	1297	309.6	1049	207.2	1137	315.2	0.27	1199	312.1	1139	341.1	1130	255.7	0.91
Protein (g)	47.4	6.507	34.27	7.619	39.97	12.07	0.07	41.35	12.67	39.62	12.95	40.31	9.436	0.94
% Protein/energy	15.14	3.018	13.12	2.142	14.14	2.183	0.20	13.82	2.21	13.97	2.054	14.43	2.663	0.68
Fat (g)	49.33	9.921	37.33	9.201	41.72	14.67	0.23	42.38	13.92	41.59	15.86	42.37	11.08	0.97
% Fat/energy	34.84	4.774	32.17	5.612	32.68	5.522	0.57	31.46	3.401	32.53	6.297	33.63	4.082	0.61
Carbohydrate (g)	166.5	53.97	143.6	34.62	150.6	42.99	0.56	161.7	39.72	151.8	47.19	147.2	36.68	0.79
% Carbohdrate/energy	50.45	6.434	54.54	6.411	53.23	5.987	0.40	54.25	5.382	53.52	6.348	52.11	5.738	0.58

Results

Obese female adolescents were screened from 2120 adolescent females and 78 of those agreed to joined this study. As shows in **Table 2**, age of the subjects were between 12-14 years old and all were obese. Data on dietary intake were calculated as an absolute number and as a relative to total energy intake. In this study, we showed that dietary fat intake of obese female adolescents was higher than dietary recommendation (< 30%). In addition, data on physical activity are presented as total physical activity and types on physical activity.

Allele frequency and Hardy-Weinberg Equilibrium (HWE) analysis are shown in **Table 3**. All allele were under HWE except GHSR rs292216. Linkage disequilibrium was calculated on *GHSR* rs292216 and rs509035 genes and a correlation was found, D = -0.099; r = -0.422; *p* = 1.6e-07. The relationship between SNPs on *GHSR* and dietary intake is shown in **Table 4**. There is no relationship between SNP on *GHSR* and dietary intake. Those analyses were supported by additional analysis using dominant, recessive, over-dominant and log additive model (data not shown).

Because the LD is significant (p < 0.05), haplotype analysis was done to evaluate the relationship between genetic variation and dietary intake indepently. In this analysis (data not shown) we found no difference between each haplotype variations on total energy, carbohydrate, fat and protein intake (*p* > 0.05). Haplotype variation of *GHSR* rs292216 and rs509035 were not related to percentage dietary carbohydrate, fat and protein to total energy intake (*p* > 0.05). There is no relationship between haplotype variation of *GHSR* rs292216 and rs509035 on high fat intake (**Figure 1**).

Discussion

In order to evaluate the influence of genetic factors on dietary intake in obese female adolescents, SNPs from *GHSR* rs292216 and rs509035 were analysed. In general, obese female adolescents consumed fat more than recommendation (30% of total energy intake). From those SNPs, we found no significant influence of SNPs in *GHSR* rs292216 and *GHSR* rs509035 on dietary intake.

GHSR has been reported as the important factor that regulate dietary intake and appetite because its function as receptor for ghrelin [10]. In a study done by de Hoed et al. [11], it was shown that subjects with A allele of GHSR gene polymorphisms (477G > A) had higher dietary restraint, disinhibition and perceived hunger. Interestingly, they also found that there was a relationship between GHSR gene polymorphism (477G > A) and postprandial PYY response [11]. PYY is peptide YY which widely known to influence dietary intake in human [12].

Although the role of GHSR on feeding behaviour has been increasingly clear, to date there is a limited data on the relationship between SNPs on GHSR on dietary intake. To our knowledge, there is no studies reporting the role of *GHSR* rs292216 and rs509035 polymorphism on dietary intake of obese female adolescents before. A study done by Wang et al. [13] showed no significant correlation between SNP in GHSR rs495225 on body weight of children and adolescents. On the other hand, Baessler et al. revealed that common SNPs and haplotypes of GHSR are involved in development of obesity.

Interestingly, although it has been suggested by Baessler et al. [9] that physiological role of GHSR gene is thorugh dietary intake, Lin et al.
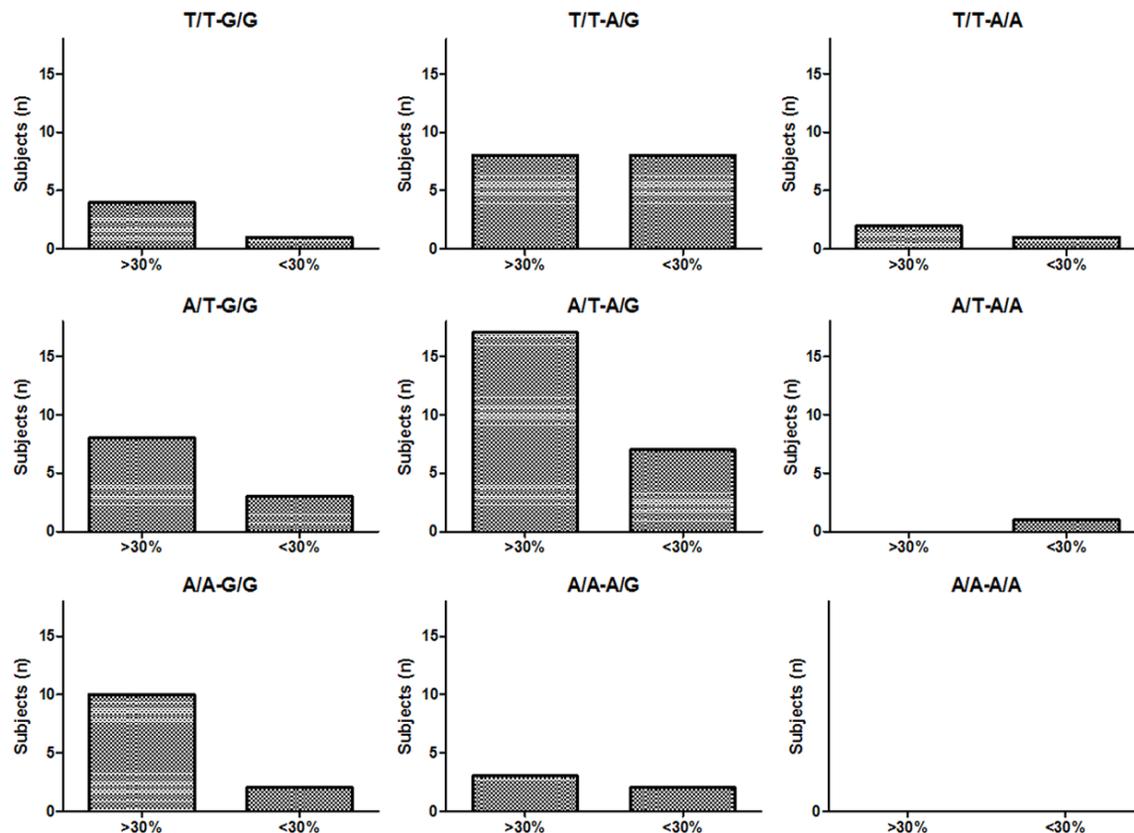
Figure 1. SNP-SNP interaction analysis on the relationship between combination of *GHSR* rs292216 and rs509035 with high fat intake (> 30%).

[14] stated that GHSR regulates apetite and satiety but not total food intake. The study compared feeding pattern of Ghsr-null mice with wild-type mice. In their study, mice with lack of Ghsr ate larger meal, took longer time to eat and ate less frequently compared to wild-type mice. This data then supported by higher expression of several neuropeptides such neuropeptide Y and Agouti-related peptide and lower expression of anoeric peptide pro-opiomelanocortin in Ghsr-null mice compared to the wild type.

We noticed several limitations in this study. First, there is a high possibility on underreporting in dietary using a 24 hours dietary recall data. Therefore, during our analysis and data presentation, we also showed the data on nutrients intake as relative to energy intake. This will give brief information regarding dietary composition of the respondents. Second, because our respondents were obese female adolescents, generalization to the overall population could not be made.

In this study, we concluded that *GHSR* rs292216 and rs509035 gene polymorphisms were not related to dietary intake in obese female adolescents. Further study is needed to investigate the effect of those genes on dietary intake and physical activity at the broader population. Studies that measure the effect of those SNPs on some mediators of dietary intake such as ghrelin and PYY are also warranted.

Disclosure of conflict of interest

None to disclose.

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References

[1] Daniels SR, Arnett DK, Eckel RH, Gidding SS, Hayman LL, Kumanyika S, Robinson TN, Scott

BJ, St Jeor S, Williams CL. Overweight in children and adolescents: Pathophysiology, consequences, prevention, and treatment. Circulation 2005; 111: 1999-2012.

[2] Dietz WH. Overweight in childhood and adolescence. N Engl J Med 2004; 350: 855-857.

[3] Indonesian Ministry of Health. Riset Kesehatan Dasar (Basic Health Surveylence) 2013.

[4] Cooper Z, Fairburn CG. Refining the definition of binge eating disorder and nonpurging bulimia nervosa. Int J Eat Disord 2003; 34 Suppl: S89-95.

[5] Adair LS, Popkin BM. Are child eating patterns being transformed globally? Obes Res 2005; 13: 1281-1299.

[6] Rodríguez G, Moreno LA. Is dietary intake able to explain differences in body fatness in children and adolescents? Nutr Metab Cardiovasc Dis 2006; 16: 294-301.

[7] Martinez JA. Body-weight regulation: causes of obesity. Proc Nutr Soc 2000; 59: 337-345.

[8] De Castro JM. Genetic influences on daily intake and meal patterns of humans. Physiol Behav 1993; 53: 777-782.

[9] Baessler A, Hasinoff MJ, Fischer M, Reinhard W, Sonnenberg GE, Olivier M, Erdmann J, Schunkert H, Doering A, Jacob HJ, Comuzzie AG, Kissebah AH, Kwitek AE. Genetic linkage and association of the growth hormone secretagogue receptor (ghrelin receptor) gene in human obesity. Diabetes 2005; 54: 259-267.

[10] Korbonits M, Goldstone AP, Gueorguiev M, Grossman AB. Ghrelin-A hormone with multiple functions. Front Neuroendocrinol 2004; 25: 27-68.

[11] De Hoed M, Smeets AJPG, Veldhorst MAB, Nieuwenhuizen AG, Bowman FG, Heidema AG, Mariman ECM, Westerterp-Plantega MS, Westerterp K. SNP analyses of postprandial responses in (an) orexigenic hormones and feelings of hunger reveal long-term physiological adaptations to facilitate homeostasis. Int J Obes 2008; 32: 1790-1798.

[12] Batterham RL, Bloom SR. The gut hormone peptide YY regulates appetite. Ann N Y Acad Sci 2003; 994: 162-168.

[13] Wang HJ, Geller F, Dempfle A, Schauble N, Hagemann S, Gortner L, Huse K, Remschmidt H, Bettecken T, Meitinger T, Schafer H, Hebebrand J, Hinney A. Ghrelin receptor gene: Identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students and children with short normal stature. J Clin Endocrinol Metab 2004; 89: 157-162.

[14] Lin L, Nuotio-Antar AM, Ma X, Lio F, Fiorotto ML, Sun Y. Ghrelin receptor regulates appetite and satiety during aging in mice by regulating meal frequency and portion size but not total food intake. J Nutr 2014; 144: 1488.