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Original Article

Overrepresentation of *GHSR* rs572169C in Long-Lived Individuals and Its Favorable Impacts on Glycolipid Modulations

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SUMMARY

Background: To explore the association between growth hormone secretagogue receptor (GHSR) rs572169 and serum lipid and glucose levels in a long-lived population to reveal its putative roles in longevity.
Methods: Participants were recruited in three groups: longevity group (LG, aged 90 and above, n = 496), offspring group (OG, aged 60–75, n = 723) and control group (CG, aged 60–75, n = 611). Genotyping of SNP rs572169 was performed by improved multiple ligase detection reaction (iMLDR). Association between the SNP and fasting blood glucose (FBG) and lipid levels were evaluated thereafter.
Results: Genotypic frequency of GHSR rs572169 showed no significant difference among groups, but females in CG exhibited higher CT/TT frequency than that of LG. Total cholesterol (TC) levels in T allele carriers of controls were higher than that in non-T carriers. T carriers exhibited higher TG levels than those of T noncarriers in the females of combined population and in OG. When BMI, FBG and lipid status were taken into account, T carriers tended to have an elevated FBG in the normal BMI subgroup of CG and in the hyperglycemic subgroup of OG and CG, an increased TG in the hyperlipidemic subgroup of OG, TC and HDL-C levels in the hyperlipidemic subgroup of CG while a lowered HDL-C in the normolipidemic subclass of LG.
Conclusions: GHSR rs572169 derived T allele is significantly enriched in average females and mainly linked to disadvantageous metabolic measures, while ancestral C allele is well maintained in long-lived families which is associated with favorable metabolic profiles and better survivorship.

1. Introduction

Growth hormone secretagogue receptor (GHSR) is a typical G protein coupled receptor with a 4.3 kb in length which is mainly distributed in the pituitary gland, hypothalamus and cardiovascular system and functions as a promoter of growth hormone (GH) secretion through ghrelin signaling pathway involving the regulation of appetite, food intake and digestion, glucose and lipid metabolism, and thus energy homeostasis as well as body stature.[1,2] The gene that codes for GHSR is mapped to chromosome 3q26.31 and is composed of 2 exons and 1 intron, in which, exon 1 encodes the 5′ untranslated region and the first to fifth transmembrane domains (TMDs), while exon 2 encodes the sixth and seventh TMDs. There are two types of GHSR, i. e., GHSR-1a and GHSR-1b due to alternative splicing.[1,3] The former contains all of the seven TMDs and has high capacity in combining with auxin. It is a signal-transducing form of the GHSR and is functionally active. The combination of GHSR-1a with ghrelin activates ghrelin-induced GH secretion and decreases food intake while the ablation of GHSR-1a eliminates GH release and increases food intake. By contrary, the precise physiological role of GHSR-1b, i. e., the truncated form of GHSR-1a, remains elucidative.[4,5]

Due to its critical roles in energy metabolism, GHSR has been linked to some age-related traits. For example, the density change of this receptor may lead to arteriosclerosis;[6] GHSR increases in adipose tissues during aging, and old *GHSR*$^{-}$/$^{-}$ knockout mice exhibit a lean and insulin-sensitive phenotype;[7–9] GHSR antagonist represses lipopolysaccharide-associated inflammatory reactions in macrophages.[10] Mutations in some residues of GHSR significantly impair its basal or constructive activity.[11,12] To date, a couple of polymorphisms on *GHSR* have been reported, some of which are modestly correlated with eating behavior, insulin resistance and obesity.[12,13] Of these, SNP rs572169 is a C > T (G > A) benign substitution at nucleotide 477 on the exon 1 of *GHSR*, resulting in a synonymous variant at protein 159 (Arg 159 Arg).[13,14] Currently, this SNP is implicated to correlate with not only metabolic traits but also complex phenotype such as lifespan in Dans.[13,15] However, these investigations are mostly from Caucasian populations, data in other ethnic groups, especially in minorities in China are scarce.

A unique Zhuang population living alongside the basin of Hongshui River in Guangxi Province of China has been known for its longevity for centuries. Many investigations aiming at the potential advantage factors which may contribute to the longevity have been carried out for decades, but no definite conclusion has been reached

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thus far. Our research group has been focusing on the genetic background of this population for 20 years. In the present study, we sought to determine the putative association of SNP rs572169 with common metabolic parameters and analyze its potential roles in the longevity of Hongshui River Area.

2. Materials and methods

2.1. Study subjects

A total of 1,830 Ethnic Zhuang residents populating the longevity region along the Hongshui River Basin in Guangxi Province, China, were enrolled in the current study, including longevous group (LG, aged 93.27 ± 3.01 yrs, n = 496), offspring group (OG, aged 59.84 ± 7.82 yrs, the first generation of the oldest olds, n = 723) and the control group (CG, aged63.42 ± 7.77 yrs, local elderly with matched ethnic and lifestyle status but without family history of longevity, n = 611). All participants were ethnic Zhuangs and were seemingly healthy, without signs of chronic or acute disorders. Subjects with a self-report history of stroke, myocardial infarction, diabetes mellitus and hypertension were ruled out. This study was approved by the Ethics Committee of Guangxi Medical University (No. 20160304-25). All subjects under investigation were given oral consents and were collected fingerprints to indicate assentation because of incapability of writing after receiving a full interpretation of the study.

2.2. Anthropometrics and epidemiological scanning

Information of social-demographic and lifestyle were collected with standardized procedures by two experienced physicians and hypertension, overweight and obesity were defined as described previously.[16,17]

2.3. Biochemical analyses

A venous blood sample of 8 mL was drawn for each participant in the morning after an overnight fasting, 2 mL of which was for DNA extraction, while the remaining was for serum separation and lipid detection. Fasting blood glucose (FBG) was checked right after the blood drawing by a blood glucose meter (Accu-Chek Active, Roche, Germany). Total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) levels were measured by enzymatic methods as described elsewhere. Hyperglycemia and dyslipidemia were defined as previous.[17]

2.4. DNA preparation and genotyping

Extraction of DNA from white blood cells was conducted according to standard protocol. Genotyping of *GHSR* rs572169 was performed by improved multiple ligase detection reaction (iMLDR), a technique basing on LDR which was recently developed by Genesky Biotechnologies Inc. (Shanghai, China). The principle of iMLDR, the PCR program, the purification of PCR products, the ligation reaction and the reading of iMLDR had been described previously.[18] Herein, the PCR primers for SNP rs572169 were F: 5′-GTG CTC CAC CCC GAC TAG CA-3′ and R: 5′-GGA ACT TCG GCG ACC TCC TCT-3′. The probe information for ligation reaction was as follow: rs572169RC, TCTCTCGGGTCAATTCGTCCTTTGGTGGTCACCAAGGGGAGG; rs572169RP, GTGAAGCTGGTCATCTTCGTCATC TTTTTTTTTTTT; and rs572169RT, TGTTCGTGGGCCGGATTAGT TGGTGGTCACCAAGGGGAGA. All primers and probes were designed and synthesized by Genesky Biotechnologies Inc.

2.5. Statistical analysis

SPSS16.0 (SPSS Inc, Chicago, IL) was used for statistics. Frequencies of allele and genotype were calculated directly. Levels of the quantitative parameters were presented as mean ± SD except TG levels were in medians (interquartile) due to non-Gaussian distribution. Chi-square test was used to evaluate the expected genotype frequencies between groups. The statistical evaluation for the categorical variables between groups was assessed by one-way ANOVA test. The association of GSHR genotypes with blood pressure, FBG, BMI and serum lipid were estimated by analysis of covariance (ANCOVA). Multiple logistic analyses with stepwise modelling were used to assess the association of blood pressure, FBG, BMI and serum lipid levels with genotypes (CC = 1, CT = 2, TT = 3) and several environment factors. All hypothetical tests were two-tailed values and a p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Epidemiological data

The clinical, demographic, and biochemical characteristics of the studied population had been described elsewhere.[18] After adjustment for age and gender, the most impressive findings in metabolic parameters include: the levels of FPG and BMI are remarkably lower in LG than that of CG and OG; the levels of TC, TG, HDL-C in LG are quite similar with that in CG, although LDL-C shows significantly higher, indicating long-lived individuals maintain better glycolipid metabolism.

3.2. Distribution of GHSR rs572169

As depicted in Figure 1, no difference is noted in the distribution of the allelic and genotypic frequency distribution of SNP rs572169 among the three groups ($p > 0.05$). However, when stratified, T allele and its corresponding genotypes (CT/TT) present more frequently in average females versus long-lived females ($p < 0.05$).

3.3. Association of GHSR rs572169 with metabolic risks

No difference has been found in FBG between T (i.e., CT and/or TT genotype) and non-T allele carriers in all groups (Figure 2A). In the control group, T genotypes (CT/TT) seem to be associated with elevated total cholesterol level versus CC genotype ($p = 0.045$, Figure 2B). While in the pooled population, T allele carriers tend to have higher TG level as compared to non-T allele bearers (i.e., CC genotype) ($p = 0.032$, Figure 2C). No significant difference was observed in LDL-C, HDL-C levels and HDL-C/LDL-C ratio between the two genotypes in all groups (Figure 2D, E and F). When gender is taken into account, males that harbor T genotypes in the control group predispose to increase fasting blood glucose in comparison to CC genotype ($p = 0.030$, Figure 3A); T allele carriers with higher TG level mainly fall in females of the entire population ($p = 0.030$, Figure 3C) and the offspring group ($p = 0.002$, Figure 3C) relative to T noncarriers. No different levels of TC, HDL-C, LDL-C and HDL-C/LDL-C ratio was noted between the two genotypes in the same gender in all groups (Figure 3B, D, E and F). After stratification by BMI and status of fasting blood glucose and lipids, individuals bearing T variant, relative to non-T carriers, are found to display a higher FBG in the normal BMI subgroup of the controls ($p = 0.032$, Figure S1 A), an elevation of TC and LDL-C in the normal BMI subgroup of LG ($p = 0.009$, Figure S1 B, and 0.014, Figure S1 E, respectively) but a reduction of TC and LDL-C in

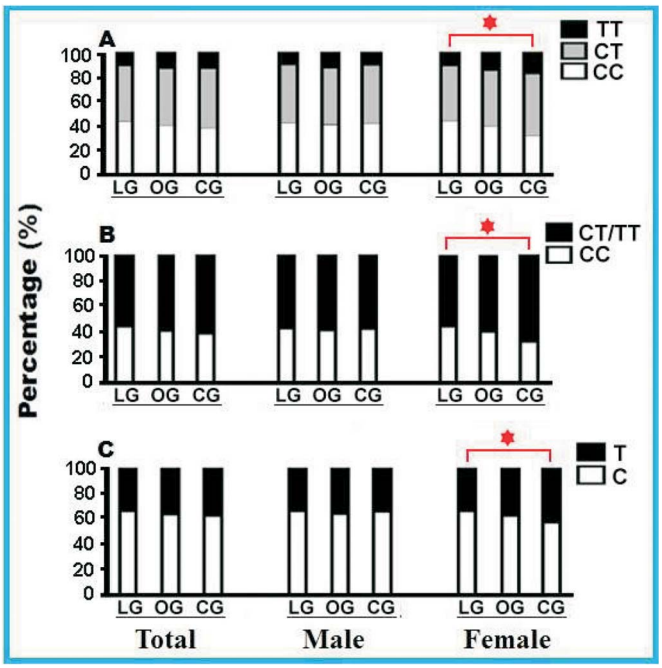

Figure 1. Distribution of genotypic and allelic frequencies of GHSR rs572169 among groups. (A) Genotypic frequency under co-dominant model, females in CG represent more TT genotype than that of LG and OG (Chi-square test, χ^2 = 10.410, p = 0.034). (B) Genotypic frequency under dominant model, females in CG represent more CT/TT genotype than that of LG and OG (Chi-square test, χ^2 = 8.451, p = 0.015). (C) Allelic frequency, females in CG represent more T allele versus LG and OG (Chi-square test, χ^2 = 10.348, p = 0.006). * p < 0.05. CG, control group; LG, long-lived group; OG, offspring group.

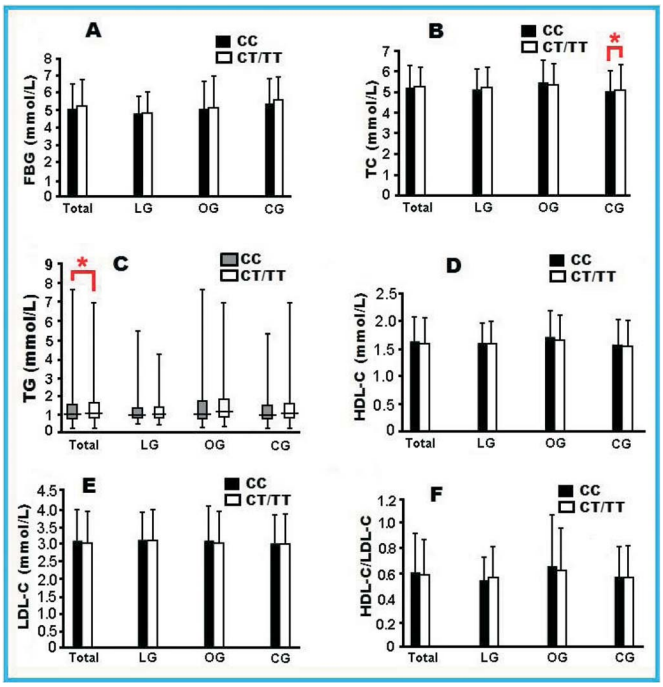

Figure 2. Overall association between GHSR rs572169 genotypes and metabolic risks. TG level is presented as Whiskers 5-95 percentile while the levels of other parameters are presented as mean ± SD. * Indicates p < 0.05. CG, control group; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LG, long-lived group; OG, offspring group; TC, total cholesterol; TG, triglyceride.

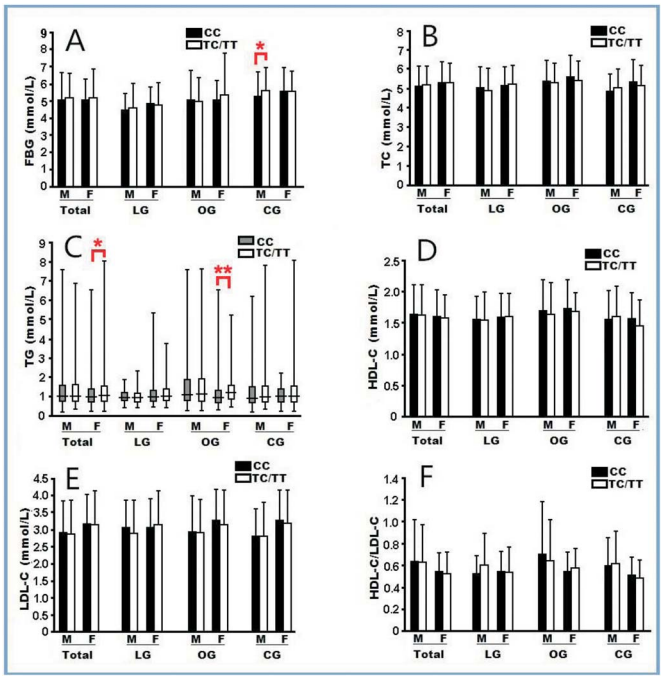

Figure 3. Association between GHSR rs572169 genotypes and metabolic risks stratified by gender. F, females; M, males. * and ** indicate p < 0.05, p < 0.01, respectively. Other notes and abbreviations see legends of Figure 2.

the overweight subgroup of LG (p = 0.015, Figure S1 B, and 0.025, Figure S1 E, respectively); a significant increment of TG in the normal BMI subgroup of total population (p = 0.007, Figure S1 C) and the control group (p = 0.030, Figure S1 C); a lowered level of FBG in the euglycemic subclass of OG (p = 0.047, Figure S2 A) and an elevated FBG level in the euglycemic subclass in CG (p = 0.047, Figure S2 A) and a higher level of LDL-C in the hyperglycemic subclass of the controls (p = 0.026, Figure S2 E); an increased TG level in the hyperlipidemic subgroup of OG (p = 0.048, Figure S3 C), an increased TC and HDL-C in the hyperlipidemic subgroup of CG (both p = 0.002, Figure S3 B and S3 D) while a lowered LDL-C in the normolipidemic subclass of LG (p = 0.041, Figure S3 E). Relative risk assessments revealed that T allele carriers in LG exhibited higher risk to be overweight (OR = 1.739, 95% CI: 1.025–2.949) but displayed no risk in developing hyperglycemia and hyperlipidemia in LG and other groups as compared to non-T carriers. Collectively, these data indicate that T allele of GHSR rs572169 may have different impact on lipid and glucose metabolism in different subpopulation under investigation and these impacts might be spontaneously influenced by different BMI, blood sugar and lipid status.

3.4. Correlation analyses

Multiple linear regression analyses showed that FBG, TC, TG, HDL-C and LDL-C correlated with, positively or negatively, different anthropometric, glycemic and lipidemic parameters in LG, OG and CG. However, no correlation was found between *GHSR* rs572169 genotypes and these metabolic risks in the three cohorts (Table S1).

4. Discussion

The first intriguing finding of the current study is the relative over-representation of the T allele of GHSR rs572169 in general population, especially in females. According to the frequecy distribution of this polymorphism and its potential correlation with unfavorable phenotypes across major ethnic groups worldwide, T allele serves predominantly as a derived and deleterious variant versus C allele under unknown evolutionary pressure.[3,13,15,19,20] This result is in line with that of Soerensen et al. who revealed that the rare allele

(T) of GHSR rs572169 was disadvatage for survival in Danish oldest old.[15] More recently, Dato and co-workers also demonstrated that *GHSR* rs572169 may interact with *MRE11A* (Meiotic Recombination 11 Homolog A) rs533984 to positively increase the survival of females, with carriers of the combination rs572169-CC/rs533984-G displaying a less mortality.[21] These data register that C allele is relatively well preserved in long-lived families as compared to average population, which may be one of the potential favarable genetic factors for their longevity. Similar observations have been noted on other loci in this studied population.[16,18,22] Thus, the mechanisms through which long-lived individuals evade the accumulation of this mutation and its resultant benefits deserve further clarification.

The second interesting result of our work is the remarkable association of this SNP with glycemic and lipidemic variables in different subpopulations, implying its putative roles in the modulation of energy homeostasis and thus in the pathogenesis of age-related phenotypes including overweight, diabetes mellitus, atherogenesis and lifespan. In particular, rs572169 T allele tends to increase FBG level in males and in subgroup with normal BMI of general elderly, to raise TG level in females in the overall population, to elevate TC and LDL-C levels in normal BMI subgroup while lower TC and LDL-C levels in overweight subgroup of LG. Together, there is a clear correlation between GHSR rs572169 and glycolipidemic metabolism but the impacts of this SNP on metabolic parameters are different among subgroups.

It is worth noting that there are everal limitations in the present work: (1) only one SNP is investigated, without data of Tag SNP or haplatypes; (2) no serum ghrelin level; (3) no survival analyses on the populations; (4) no interaction analyses on this variant with SNPs on other genes or with environmental factors. All these aspects will be taken into consideration in our further research on this long-lived population.

In conclusion, the derived allele (T) of GHSR rs572169 is more prevalent among ordinary population relative to longevous families and correlates with several deleterious glycose and lipid profiles, which might be central to their poorer survivorship. Or in other words, the ancestral allele C of this SNP is better maintained in the long-lived cohort and their offspring which might partially interpret their longevity. More variants in other loci and their interplays among genes and with environmental varibles deserves further clarification.

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Disclosure statement

The authors declare no conflict of interest.

Supplementary materials

Supplementary materials for this article can be found at http://www.sgecm.org.tw/ijge/journal/view.asp?id=22.

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