DOI 10.1007/s00702-005-0393-2
J Neural Transm (2006) 113: 1279–1285

Journal of Neural Transmission
Printed in Austria

Association of ghrelin receptor gene polymorphism with bulimia nervosa in a Japanese population

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Received July 20, 2005; accepted October 9, 2005
Published online December 16, 2005;

Summary. Eating disorders (EDs) have a highly heterogeneous etiology and multiple genetic factors might contribute to their pathogenesis. Ghrelin, a novel growth hormone-releasing peptide, enhances appetite and increases food intake, and human ghrelin plasma levels are inversely correlated with body mass index. In the present study, we examined the 171T/C polymorphism of the ghrelin receptor (growth hormone secretagogue receptor, GHSR) gene in patients diagnosed with EDs, because the subjects having ghrelin gene polymorphism (Leu72Met) was not detected in a Japanese population, previously. In addition, β3 adrenergic receptor gene polymorphism (Try64Arg) and cholecystokinin (CCK)-A receptor (R) gene polymorphism (−81A/G, −128G/T), which are both associated with obesity, were investigated. The subjects consisted of 228 Japanese patients with EDs [96 anorexia nervosa (AN), 116 bulimia nervosa (BN) and 16 not otherwise specified (NOS)]. The age- and gender-matched control group consisted of 284 unrelated Japanese subjects. The frequency of the CC type of the GHSR gene was significantly higher in BN subjects than in control subjects ($\chi^2 = 4.47$, $p = 0.035$, odds ratio = 2.05, Bonferroni correction: $p = 0.070$), while the frequency in AN subjects was not different from that in controls. The distribution of neither β3 adrenergic receptor gene nor CCK-AR polymorphism differed between EDs and control subjects. Therefore, the CC type of GHSR gene polymorphism (171T/C) is a risk factor for BN, but not for AN.

Keywords: Ghrelin, GHSR, polymorphism, eating disorder, gene.

Introduction

Ghrelin, a novel growth hormone-releasing peptide, was originally isolated from the rat and human stomachs (Kojima et al., 1999). Both intracerebroventricular and peripheral administration of ghrelin causes adiposity

by increasing food intake and decreasing fat oxidation in rodents (Asakawa et al., 2001; Nakazato et al., 2001; Tshöp et al., 2000; Wren et al., 2000). Ghrelin is also known to enhance appetite and increase food intake in healthy men (Wren et al., 2001), and human ghrelin plasma levels are inversely correlated with body mass index (BMI) (Ariyasu et al., 2001). Plasma ghrelin levels are regulated by changes in energy balance, i.e., fasting increases circulating ghrelin concentrations while feeding decreases them. In recent reports (Ariyasu et al., 2001; Otto et al., 2001), extremely high levels of ghrelin were observed in patients with anorexia nervosa (AN); weight gain decreases elevated plasma ghrelin concentrations. The increased ghrelin secretion in anorexia might reflect a physiological effort to compensate for the lack of nutritional intake and stored energy. The human ghrelin gene has been analyzed in several association studies on obesity, but inconsistent results have been obtained to date (Hinney et al., 2002; Ukkola et al., 2001, 2002). Based upon the reports by Ukkola et al. (2002) that Met 72 carrier status of the single nucleotide polymorphism (SNP) of ghrelin gene (Leu72Met) is protective against fat accumulation, we previously investigated this polymorphism. However, no subject with this polymorphism of ghrelin (Leu72Met) has been detected in a Japanese population ($n = 230$) (data not shown), although Ukkola et al. (2002) reported that 12 of 96 subjects were heterozygote. The discrepancy between the previous study (Ukkola et al., 2002) and ours might be due to racial differences.

The discovery of ghrelin was rooted in the search for an endogenous ligand for the growth hormone (GH) secretagogue receptor (GHSR). In a recent report (Wang et al., 2004), seven sequence variants of the GHSR gene were identified. Although no conclusive evidence has yet been reported for the involvement of the GHSR gene in body weight regulation, the frequency of the 171T allele of rs495225 was slightly, but not significantly, higher in obese subjects than in underweight individuals ($p = 0.14$) (Wang et al., 2004). Bulimia nervosa (BN) and AN are eating disorders (EDs) characterized by aberrant patterns of eating behavior and weight regulation.

EDs have a highly heterogeneous etiology and multiple genetic factors might contribute to their pathogenesis (Koizumi et al., 2004; Matsushita et al., 2002, 2004). In the present study, we examined the SNP of GHSR (rs495225: 171T/C, dbSNP; National Center for Biotechnology Information (NCBI): http://www.ncbi.nlm.nih.gov) in patients diagnosed with EDs. In addition, it has been found in a Japanese population that β3 adrenergic receptor gene polymorphism (Try64Arg) is associated with high BMI and obesity (Shimokata et al., 2000; Yoshida et al., 1995). We previously reported that cholecystokinin (CCK)-A receptor (R) gene polymorphism (−81A/G, −128G/T) is relevant in panic disorders and that the −81G allele is a risk factor for contractile dysfunction associated with alcoholism in a Japanese population (Miyasaka et al., 2004a, b). CCK is a gastrointestinal hormone as well as a neurotransmitter and CCK-AR mediates satiety. Subjects with the −81G/G, −128T/T genotype showed higher body fat and higher plasma leptin levels than others (Funakoshi et al., 2000). In addition, As the classification of BN and AN depends primarily on levels of minimal body weight (American Psychiatric Association, 1994), CCK-AR and β3 adrenergic receptor gene polymorphisms were also investigated in ED patients.

Materials and methods

This study was approved by the Ethics Committees of the Kurihama Alcoholism Center and the Tokyo Metropolitan Institute of Gerontology. All human studies have been performed in accordance with the ethical standards stated in the appropriate version of the 1964 Declaration of Helsinki. Written informed consent was obtained from each subject.

Subjects

The subjects were 228 Japanese patients with EDs (16 males, 21–35 years old; 212 females, 19–69 years old) who had been diagnosed, evaluated and treated at one of three hospitals (Kurihama Alcoholism Center, Akagi Kougen Hospital or Kobe University Hospital). Information on clinical parameters was obtained by three of the authors (S.M., K.S., and S.H.) in face-to-face interviews, using a clinical assessment form developed for this study. The criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (American Psychiatric Association, 1994) were used to diagnose EDs. Clinical data evaluated were 1) family history of EDs and other mental disorders; 2) age at onset and course of the disorder, including changes in height and weight; and 3) DSM-IV Axis I disorders other than EDs and Axis II disorders. Our subjects had been diagnosed with AN (n = 96), BN (n = 116) or EDs not otherwise specified (NOS, n = 16). AN patients were classified into two subtypes: the restricting type (ANR: n = 47) and the binge-eating and/or purging type (ANP: n = 49). Likewise, BN patients were also classified into two subtypes: the purging type (BNP: n = 91) and the non-purging type (BNNP: n = 25). The age- and gender-matched control group consisted of 284 unrelated Japanese subjects (26 males, 19–52 years old; 258 females, 18–43 years old). The controls were employees and students at the Kurihama Alcoholism Center and in the Tokyo Metropolitan Institute of Gerontology. Controls were also asked based on the results of the Eating Attitudes Test (EAT-26) (Garner et al., 1982). No control subject showed any signs of abnormal eating behavior.

Genotyping

Genomic DNA was extracted from peripheral leukocytes, and rs495225 (171C to T) in the coding region of the GHSRs were examined following the method described by Wang et al. (2004). Examination of the polymorphism of the GHSR gene was accomplished using a mismatch polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Wang et al., 2004). Briefly, a pair of primers (sense primer = 5′-CGGGGTTCAACCTCACACT-3′; antisense primer = 5′-AGAGCGCACCGCAAACTC-3′) were used to amplify the 593-bp product, which was subsequently digested with restriction enzyme LweI (ER1621) and fractionated by electrophoresis on a 3.5% agarose gel in Tris-borate buffer. The PCR products were isolated and we confirmed that each of the genotypes showed the exact sequences of the GHSR gene.

β3 adrenergic receptor polymorphism (Trp64Arg) and CCK-AR polymorphism (−81A/G, −128G/T) were determined according to the methods previously reported (Funakoshi et al., 2000; Shimokata et al., 2000).

Statistical analysis

Statistical differences were assessed by 2×2 chi-square test. An odds ratio with a 95% confidence interval (CI) was calculated to evaluate the genotype frequencies between groups. In some case, to avoid multiple testing, the effect of GHSR gene polymorphism (171T/C) on BN, AN, and EDs was assessed using logistic regression method controlling for age and gender. Probability differences of $p < .05$ were considered to be statistically significant.

Results

A separate NOS group was excluded, first, because it was too small and underpowered to provide any useful information. Examination of the clinical characteristics of the patients revealed that alcohol dependence or abuse was the most common, existing in 50.9% of cases in patients with BN, compared with 26.0% in patients with AN. Approximately one quarter of the patients with EDs had comorbid borderline personality disorder (25.9% for BN, 17.7% for AN). Depressive status was observed in 9.5% of cases in patients with BN and 4.2% in patients with AN. These percentages were similar to a previous report (Nishiguchi et al., 2001).

Table 1 shows the genotype frequencies of GHSR gene 171T/C polymorphism in patients with EDs and control subjects. The genotype distribution in the two groups did not deviate significantly from the Hardy-Weinberg equilibrium. The frequency of the CC type in ED subjects was slightly higher than in controls, but did not reach statistical significance. Separate examination by disorder revealed that in BN patients, the CC type occurred significantly more frequently than in control subjects ($\chi^2 = 4.47$, $p = 0.035$, odds ratio = 2.05, Bonferroni correction: $p = 0.070$), while the frequency in AN patients did not differ from that in control subjects (Table 1). When analyzed by the

Table 1. Genotype and allele frequencies of GHSR gene 177T/C polymorphism in patients with eating disorders and control subjects

Group	n	Genotype		
		TT	TC	CC
Controls	284	129 (45.4)	133 (46.8)	22 (7.7)
Eating disorders (total)	228	110 (48.2)	91 (39.9)	27 (11.8)
Anorexia nervosa (AN)	96	50 (52.1)	37 (38.5)	9 (9.4)
Restricting type (ANR)	47	24 (51.1)	21 (44.7)	2 (4.2)
Binge-eating and/or purging type (ANP)	49	26 (53.1)	16 (32.7)	7 (14.3)
Bulimia nervosa (BN)	116	50 (43.1)	49 (42.2)	17 (14.7)*
Purging type (BNP)	91	43 (47.3)	35 (38.5)	13 (14.3)
Non-purging type (BNNP)	25	7 (28.0)	14 (56.0)	4 (16.0)
Eating disorders not otherwise specified (NOS)	16	10 (62.5)	5 (31.3)	1 (6.3)

Numbers in parentheses indicate percentages. The difference between the CC type and TT and TC types was tested by 2×2 chi-square test. *Significantly different from control subjects ($\chi^2 = 4.47$, d.f. $= 1$, $p = 0.035$, odds ratio $= 2.05$)

logistic regression method, the frequency of subjects who had the CC genotype was significantly higher in BN than in controls ($p = 0.0294$; 95% CI, 1.088–4.935).

Although the frequency of the CC type was lower in the ANR group than in other subgroups (the sum of ANP, BNP and BNNP), the difference was not significant ($\chi^2 = 3.60$, $p = 0.058$) (Tabel 1). The ED subtypes can be characterized as having the common clinical feature of binge-eating and/or purging, and patients with ED are often associated with depression, alcoholism and/or borderline personality. When the frequency of the CC type was compared between ED patients with binge-eating and/or purging (ANP + BNP) and those without purging (ANR + BNNP), there were no significant differences ($\chi^2 = 1.83$, $p = 0.21$). The distribution of GHSR gene 171T/C

Table 2. Genotype of the β3-adrenergic receptor gene Trp64Arg polymorphism in patients with eating disorders and control subjects

Group	n	Genotype	
		TT	TA + AA
Controls	282†	168 (59.6)	114 (40.4)
Eating disorders (total)	228	142 (62.3)	86 (37.7)
Anorexia nervosa (AN)	96	67 (69.8)	29 (30.2)
Restricting type (ANR)	47	33 (70.2)	14 (29.8)
Binge-eating and/or purging type (ANP)	49	34 (69.4)	15 (30.6)
Bulimia nervosa (BN)	116	66 (56.9)	50 (43.1)
Purging type (BNP)	91	50 (54.9)	41 (45.1)
Non-purging type (BNNP)	25	16 (64.0)	9 (36.0)
Eating disorders not otherwise specified (NOS)	16	9 (56.3)	7 (43.8)

Numbers in parentheses indicate percentages. †Two samples could not be examined, thus, the total number was 282 rather than 284. *TT* Trp64Trp (wild-type); *TA* Trp64Arg; *AA* Arg64Arg

polymorphism was not associated with any clinical features ($p = 0.171$ for association with depression; $p = 0.641$, alcoholism; and $p = 0.637$, borderline personality). The age of onset of EDs was not different regardless of 171C/T genotype (17.8 ± 3.3 years old, mean $\pm$ SD, for the CC type; 18.7 ± 4.7 for the TC type; and 18.1 ± 5.0 for the TT type).

Table 2 shows the genotype frequencies of β3 adrenergic receptor gene polymorphism (Trp64Arg). The frequency of Trp64Arg (TA) and Arg74Arg (AA) in AN patients tended to be lower than in control subjects, however, the difference was not statistically significant ($\chi^2 = 3.179$, $p = 0.075$). The difference between AN and BN subjects was also not significant ($\chi^2 = 3.736$, $p = 0.053$) (Table 2).

The distribution of CCK-AR gene polymorphism did not differ between ED and control subjects; the frequencies of the AA/GG type were 59.2% for control subjects and 58.3% for ED subjects (data not shown). As the distribution of CCK-AR gene polymorphism is not associated with EDs, the combination of GHSR gene polymorphism and β3 adrenergic receptor gene polymorphism was estimated. The frequencies of subjects with both the 171CC type of GHSR gene polymorphism and β3 adrenergic receptor polymorphism (heterozygous and homozygous) showed no significant differences, at 10 of 284 control subjects (3.5%) and 9 of 228 ED subjects (3.9%).

Discussion

The present study first demonstrates the association of GHSR gene 171T/C polymorphism with BN, but not with AN. In contrast with a recent report by Wang et al. (2004) of a weak (not statistically significant) association of the 171T allele with obesity, we observed a positive association of the CC genotype with BN. This difference in results may be due to different subjects: Wang et al. (2004) investigated healthy subjects, while the present subjects were ED patients. In the present study, as the frequency of the CC type was lower in the ANR group than in other subgroups (ANP + BNP + BNNP), $\chi^2 = 3.60$, $p = 0.058$), a weak association of the 171CC genotype with the phenotype ''over eating'' may be proposed.

It is suggested that loss-of-function mutations in GHSR may potentially entail underweight status and/or a decrease in GH and ghrelin secretion leading to leanness, and that gain-of-function mutations could lead to obesity. However, the polymorphism of 171T/C occurs within the transmembrane domain and does not alter the amino acid sequence. The fact that the 171T/C polymorphism has no effect on the aminoacid sequence suggests that it is unlikely to be functional. Thus, linkage dysequilibrium with a functional polymorphism is likely to be involved in the underlying mechanism. More recently, Baessler et al. (2005) reported a linkage among five SNPs (rs509035, rs572169, rs519384, rs512692 and rs863441), their haplotypes and BMI, although 171T/C polymorphism was not investigated. The possibility that these haplotypes might be relevant to EDs, could not be excluded. Further studies of different SNPs including GHSR in family based studies pertaining EDs should be conducted.

In a recent report (Tanaka et al., 2003), it was observed that plasma ghrelin levels were higher in ANP than in ANR, although the precise mechanism remains unknown. In the present study, although we did not measure plasma ghrelin levels, we found no connection between GHSR171T/C polymorphism and the presence or absence of binging/purging in ED patients.

The β3 adrenergic receptor has been linked to the regulation of lipolysis and thermogenesis (Umekawa et al., 1999), and subjects with this polymorphism tend to show higher body weight. Therefore, we expected a higher frequency of β3 adrenergic receptor gene polymorphism in BN subjects. Indeed, the frequency of β3 adrenergic receptor gene

polymorphism tended to be lower in AN than in BN, but the difference did not reach statistical significance ($p = 0.053$). CCK-AR gene polymorphism has been linked to an increase in body fat (Funakoshi et al., 2000) and the combination of CCK-AR gene polymorphism and β3 adrenergic receptor gene polymorphism is known to be associated with severe weight gain in Japanese males (Koda et al., 2004). However, these polymorphisms showed no association with EDs. In conclusion, the 171CC type of GHSR gene polymorphism is a risk factor for BN but not for AN.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research B-15390237 (to K.M.), and for Longevity Sciences from the Ministry of Health and Welfare 12-01 (to A.F.).

References

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th ed. American Psychiatric Press, Washington, DC

Ariyasu H, Takay K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K (2001) Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 86: 4753–4758

Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino A, Kasuga M (2001) Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology 120: 337–345

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

Funakoshi A, Miyasaka K, Matsumoto H, Yamamori S, Takiguchi S, Kataoka K, Takata Y, Matsusue K, Kono A, Shimokata H (2000) Gene structure of human cholecystokinin (CCK) type-A receptor: body fat content is related to CCK type-A receptor gene promoter polymorphism. FEBS Lett 466: 264–266

Garner DM, Olmsted MP, Bohr Y, Garfinkel PE (1982) The eating attitudes test: psychometric features and clinical correlates. Psychol Med 12: 871–878

Hinney A, Hoch A, Geller F, Schäfer H, Siegfried W, Goldschmidt H, Remschmidt H, Hebebrand J (2002) Ghrelin gene: identification of missense variants and a frameshift mutation in extremely obese children and adolescents and healthy normal weight students. J Clin Endocrinol Metab 87: 2716–2719

Koda M, Ando F, Niino N, Shimokata H, Miyasaka K, Funakoshi A (2004) Association of cholecystokinin 1 receptor and β3-adrenergic receptor polymorphisms with midlife weight gain. Obesity Res 8: 1212–1216

Koizumi H, Hashimoto K, Itoh K, Nakazato M, Himizu E, Ohgake S, Koike K, Okamura N, Matsushita S, Suzuki K, Murayama M, Higuchi S, Iyo M (2004) Association between the brain-derived neurotrophic factor 196GA polymorphism and eating disorders. Am J Med Genet 127B: 125–127

Kojima M, Hosoda H, Date Y, Nakazato M, Kangawa K (1999) Ghrelin is a growth-hormone releasing acylated peptide from stomach. Nature 402: 656–660

Matsushita S, Nakamura T, Nishiguchi N, Higuchi S (2002) Association of serotonin transporter regulatory region polymorphism and abnormal eating behaviors. Mol Psychiat 7: 538–540

Matsushita S, Suzuki K, Murayama M, Nishiguchi N, Hishimoto A, Takeda A, Shirakawa O, Higuchi S (2004) Serotonin transporter regulatory region polymorphism is associated with anorexia nervosa. Am J Med Genet 128B: 114–117

Miyasaka K, Yoshida Y, Matsushita S, Higuchi S, Maruyama K, Niino N, Ando F, Shimokata H, Ohta S, Funakoshi A (2004a) Association of cholecystokinin-A receptor gene polymorphism with alcohol dependence in a Japanese population. Alco and Alco 39: 25–28

Miyasaka K, Yoshida Y, Matsushita S, Higuchi S, Shirakawa O, Shimokata H, Funakoshi A (2004b) Association of cholecystokinin-A receptor gene polymorphisms and panic disorder in Japanese. Am J Med Genet 127B: 78–80

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S (2001) A role for ghrelin in the central regulation of feeding. Nature 409: 194–198

Nishiguchi N, Matsushita S, Suzuki K, Murayama M, Shirakawa O, Higuchi S (2001) Association between 5HT2A receptor gene promoter region polymorphism and eating disorders in Japanese patients. Biol Psychiatry 50: 123–128

Otto B, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, Heiman MI, Lehnert P, Fiochter M, Tschöp M (2001) Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. Eur J Endocrin 145: R5–R9

Shimokata H, Yamada Y, Nakagawa M, Okubo R, Saido T, Funakoshi A, Miyasaka K, Ohta S, Tsujimoto G, Tanaka M, Ando F, Niino N (2000) Distribution of geriatric disease-related genotypes in the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidem [Suppl] 10: 46–55

Tanaka M, Naruo T, Yasuhara D, Tatebe Y, Nagai N, Shiiya T, Nakazato M, Matsukura S, Nozoe S (2003) Fasting plasma ghrelin levels in subtypes of anorexia nervosa. Psychoneuroendocrinol 28: 829–835

Tshöp M, Smiley DL, Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature 407: 908–913

Ukkola O, Ravussin E, Jacobson P, Pérusse L, Rankinen T, Tschöp M, Heiman ML, Leon AS, Rao DC, Skinner JS, Wilmore JH, Sjöström L, Bouchard C (2002) Role of ghrelin polymorphisms in obesity based on three different studies. Obes Res 10: 781–791

Ukkola O, Ravussin E, Jacobson P, Snyder EE, Chagnon M, Sjöström L, Bouchard C (2001) Mutations in the preproghrelin/ghrelin gene associated with obesity in humans. J Clin Endocrinol Metab 86: 3996–3999

Umekawa T, Yoshida T, Sakane N, Kogure A, Kondo M, Honjo H (1999) Trp64Arg mutation of β3-adrenoceptor gene deteriorates lipolysis induced by β3-adrenoceptor agonist in human omental adipocytes. Diabetes 48: 117–120

Wang HJ, Geller F, Demplle A, Achäuble N, Friedel S, Lichtner P, Fontenla-Horro F, Wudy S, Hagemann S, Gortner L, Huse K, Remschmidt H, Bettechken T, Meitinger T, Schäfer H, Hebebrand J, Hinney A (2004) 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 80: 157–162

Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR (2001) Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 86: 5992–5995

Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Robers GH, Morgan DG, Ghatai MA, Bloom SR (2000) The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141: 4325–4328

Yoshida T, Sakane N, Umekawa T, Sakai M, Takahashi T, Kondo M (1995) Mutation of β3-adrenergic-receptor gene and response to treatment of obesity. The Lancet 346: 1433–1434

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