Deletion of Gpr128 results in weight loss and increased intestinal contraction frequency

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Abstract

AIM: To generate a Gpr128 gene knockout mouse model and to investigate its phenotypes and the biological function of the Gpr128 gene.

METHODS: Bacterial artificial chromosome-retrieval methods were used for constructing the targeting vector. Using homologous recombination and microinjection technology, a Gpr128 knockout mouse model on a mixed 129/BL6 background was generated. The mice were genotyped by polymerase chain reaction (PCR) analysis of tail DNA and fed a standard laboratory chow diet. Animals of both sexes were used, and the phenotypes were assessed by histological, biochemical, molecular and physiological analyses. Semi-quantitative reverse transcription-PCR and Northern blotting were used to determine the tissue distribution of Gpr128 mRNA. Beginning at the age of 4 wk, body weights were recorded every 4 wk. Food, feces, blood and organ samples were collected to analyze food consumption, fecal quantity, organ weight and constituents of the blood and plasma. A Trendelenburg preparation was utilized to examine intestinal motility in wild-type (WT) and Gpr128-/- mice at the age of 8 and 32 wk.

RESULTS: Gpr128 mRNA was highly and exclusively detected in the intestinal tissues. Targeted deletion of Gpr128 in adult mice resulted in reduced body weight gain, and mutant mice exhibited an increased frequency of peristaltic contraction and slow wave potential of the small intestine. The Gpr128-/- mice gained more weight on average than the Gpr128+/- mice since 24 wk, being 30.81 ± 2.84 g and 25.74 ± 4.50 g, respectively (n = 10, P < 0.01). The frequency of small intestinal peristaltic contraction was increased in Gpr128-/- mice. At the age of 8 wk, the frequency of peristalsis with an intraluminal pressure of 3 cmH2O was 6.6 ± 2.3 peristalsis/15 min in Gpr128+/- mice. At the age of 8 wk, the frequency of peristalsis with an intraluminal pressure of 3 cmH2O was 6.6 ± 2.3 peristalsis/15 min in Gpr128+/- mice. At the age of 32 wk, the frequency of peristaltic contraction with an intraluminal pressure of 2 and 3 cmH2O was 4.6 ± 2.3 and 3.1 ± 0.8 peristalsis/15 min in WT intestine (n = 8), whereas in Gpr128-/- mice (n = 8) the frequency of contraction was 8.3 ± 3.0 and 7.4 ± 3.1 peristalsis/15 min, respectively (2 cmH2O: P < 0.05 vs WT; 3 cmH2O: P < 0.01 vs WT). The frequency of slow wave potential in Gpr128+/- intestine (35.8 ± 4.3, 36.4 ± 4.2 and 37.1 ± 4.8/min with an intraluminal pressure of 1, 2 and 3 cmH2O, n = 8) was also higher than in...
We have generated a mouse model with a targeted deletion of Gpr128 and found reduced body weight and increased intestinal contraction frequency in this animal model.

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Key words: G-protein-coupled receptors; Gpr128; Knockout mouse; Weight loss; Intestinal contraction frequency

Core tip: The Adhesion family is the second largest subfamily of the G-protein-coupled receptors (GPCR). The physiological function of the orphan Adhesion-GPCR Gpr128 is unknown. In the present study, we generated Gpr128 knockout mice and confirmed the selective expression of Gpr128 in the intestinal tissues. Phenotypic analysis revealed that targeted deletion of Gpr128 in the mouse resulted in reduced body weight gain and increased frequency of peristaltic contraction and slow wave potential in the small intestine. The physiological roles of Gpr128 in the gastrointestinal tract and its potential as a therapeutic target for obesity and nutritional disorders warrant further investigation.

INTRODUCTION

G protein-coupled receptors (GPCRs) constitute one of the largest protein families in humans[1,2] and play important roles in the transduction of intercellular signals across the plasma membrane via different G-proteins[3,4]. GPCRs respond to a large variety of extracellular signals including small molecules such as Ca^{2+}, hormones, peptides, chemokines and other factors as well as sensory stimuli such as vision, smell, taste and neuronal transmission in response to photons[5]. Due to their extremely diverse roles in biological processes, GPCRs represent important molecular targets for biomedical research and drug discovery[6].

The adhesion family of GPCRs (Adhesion-GPCRs) is the second largest subfamily of GPCRs, with over 30 members found in mammals[7,8]. These proteins are characterized by the dual presence of a secretin-like seven-transmembrane (7TM) domain and a long cell adhesion-like N-terminal domain, which typically consists of a functional GPCR proteolytic site domain (GPS domain) and one or more conserved domains[9,10]. Generally, the long N-termini bind various proteins that promote cell-to-cell and cell-to-matrix interactions[11]. However, some Adhesion-GPCRs were found to have a GPS domain but to lack the conserved domains. HE6 and GPR56 are two such members for which no N-terminal conserved domains have been identified, although they have both been shown to have adhesive properties. HE6 attachment appeared to be required for the maturation of germ cells because mutation of this receptor resulted in male infertility in mice[12]. Mutations in GPR56 have been shown to be associated with cortical malformation of the human brain[13,14] and to participate in tumor cell adhesion[15,16].

GPR128 is an orphan receptor of the Adhesion-GPCR family of the G-protein-coupled receptors (GPCR). The tissue distribution of GPR128 in mammals. Using PCR, Northern blotting and immunofluorescence staining, we show that Gpr128 might be exclusively expressed in mouse intestine tissue. To study the role of Gpr128 in the intestine, we generated mice with a targeted deletion of Gpr128. We found that Gpr128 knockout mice exhibited less body weight gain and an increase in intestinal contraction frequency compared with their wild-type (WT) counterparts.

MATERIALS AND METHODS

Construction of the Gpr128 targeting vector and electroporation of embryonic stem cells

The 129/Sv bacterial artificial chromosome (BAC) clone bMQ-239c21 was provided by the Sanger Institute. BAC-retrieval methods were used for constructing the targeting vector[22,23].

The sequence, including the GPS domain and a portion of the 7TM domain, was retrieved from the BAC clone using a retrieval vector containing two homologous arms.

A targeting vector was constructed by replacing the mouse Gpr128 genomic fragment (8.4 kb) covering exons 10-12 with the 1.9-kb phosphoglycerate kinase-neomycin resistance (PGK-Neo) cassette for positive selection and was laid with an external herpes simplex virus-1-thymi-
the targeting vector was electroporated into embryonic as homologous recombination arms. After linearization, the targeting vector was electropropared into embryonic stem (ES) cells derived from 129/Sv G418- and GANC-resistant clones were selected using two pairs of PCR primers. The sequences of the primers used for identifying the recombinant clones are as follows: 5'-CCATAGGAGAATAATATCAACAATC-3' (forward primer P1), 5'-CTGAGCCAGAAGCAGGAAGGA-3' (reverse primer P2), 5'-ACAAAAAGCAAAAAGGTTCTGGAAG-3' (forward primer P3) and 5'-CTCCCCCGTGGCTTCCCTTGAC-3' (reverse primer P4).

Generation of Gpr128 knockout mice

Chimeric male mice were generated by injecting the recombinant ES cell clone into C57BL/6 blastocysts, which were subsequently implanted into pseudopregnant female recipient mice. Germ line transmission was monitored by crossing chimeric mice with WT 129/Sv female mice and selected for sib mating to create WT (Gpr128+/+), heterozygous (Gpr128+/−) and homozygous mice (Gpr128−/−) for further experiments.

The mice were genotyped by PCR analysis of tail DNA using two primer pairs, which allows the amplification of WT and targeted alleles. The forward primer P3 and reverse primer P4 were used to amplify the 3’ targeted allele, which yields a 5.7 kb band. The length of the WT allele is 5.4 kb.

Semi-quantitative RT-PCR

All experiments involving animals were conducted under protocols approved by Institutional Animal Care and Use Committee of Shanghai Research Center for Model Organisms (Approval ID: 2010-0017), and the care of animals was in accord with the institution’s guidelines.

The mice were anesthetized with ketamine and xylazine diluted in 0.9% saline, and all efforts were made to minimize animal suffering. Total RNA was extracted from adult mouse tissues using Trizol reagent (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's instructions. Northern blotting was performed as described in the manual provided by the manufacturer (Northern Max-Gly; Ambion Inc., Carlsbad, CA, United States). A 1-µg aliquot was removed from each mRNA sample for adult WT mice for analysis. The probe used for Gpr128 was a 715-bp DNA fragment prepared from mouse intestine cDNA using the PCR forward primer N1 (5’-AGAGTCGAGACACAGACACCAGCTGAGGGAAG-3’) and reverse primer N2 (5’-TGGCACTCTACTGGAAGGGAG-3’). Probe DNA (25 ng) was labeled with [α-32P]-dATP using a Random Primer Labeling Kit (NEBlot Kit, NEB, Beverly, MA, United States) and subsequently purified by gel filtration.

Maintenance and body weight studies of Gpr128-deficient mice

All mice used in this study were on a mixed 129/BL6 background. The mouse colony was maintained in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle, and the mice were fed a standard laboratory chow diet with free access to water. The animals were maintained by crossing heterozygous progeny.

Beginning at the age of 4 wk, body weights were recorded every 4 wk. Animals of both sexes were used, but littersmates were matched by gender.

Histology and immunofluorescence staining

The intestines of WT and Gpr128−/− mice at 8 wk of age were collected and fixed with 10% formalin for sectioning followed by hematoxylin and eosin (HE) staining. Sections (6 mm) were cut and stained with HE according to standard procedures. For immunofluorescence analysis, paraffin-embedded sections were deparafinized with xylene and treated with gradually decreasing concentrations of ethanol. The sections were blocked for 1 h in 5% bovine serum followed by staining overnight at 37 °C with goat anti-GPR128 antibodies (sc-48208, Santa-Cruz Biotechnology Inc., Santa-Cruz, CA, United States) for human and mouse tissues and finally incubated with fluorescent-conjugated secondary antibody for 30 min. Finally, the slides were rinsed with PBS and mounted with VECTASHIELD mounting medium (H-1200, Vector Laboratories Inc., Burlingame, CA, United States).

Food consumption studies and fecal quantity analysis

At week 16 of the experimental diet period, the mice were
individually caged and given preweighed food for 5 d. During this period, the amount of food consumed was determined, and feces were quantitatively collected over a 24 h period. The results are expressed as grams of food consumed and feces excreted per day.

**Analyses for the constituents of the blood and plasma**

After the 32 wk experimental feeding period, the mice were fasted for 16 h and subsequently anesthetized with ketamine and xylazine diluted in 0.9% saline. Blood was removed by cardiac puncture into tubes containing 1 mmol/L EDTA. White adipose (epididymal and uterine fat pads) and brown adipose (intrascapular) tissue as well as the heart, liver, spleen, lungs, and kidneys were removed, and the wet weight of each was recorded.

Blood samples were collected for complete blood counts including white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, white small cell rate, white middle cell rate, and white large cell rate using an automated hematology analyzer (Poch-100ivd, Sysmex, Kobe, Japan). Plasma was obtained by low-speed centrifugation of the blood samples for measurement of albumin/globulin, globulin, low-density lipoprotein cholesterol, albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, urea nitrogen, creatinine, glucose, high-density lipoprotein cholesterol, lactate dehydrogenase, total cholesterol, triglycerides and total protein using an automated chemistry analyzer (CHEMIX-180; Sysmex, Kobe, Japan).

**Analysis of intestine motility**

Male and female mice at 8 and 32 wk of age were sacrificed. A Trendelenburg preparation was utilized to examine intestinal motility in WT and Gpr128<sup>−/−</sup> mice. Briefly, the jejunum was removed and placed in pre-oxygenated Kreb's Ringer solution at room temperature. A segment of the jejunum (6 cm long) was placed into an organ bath and analyzed using the Spike2 program (CED, United Kingdom). The preparation was allowed to stabilize for at least 40 min before the experiments were started.

**Statistical analysis**

The data are presented as the mean ± SD. Differences between groups were determined by the 2-tailed Student t test. P values less than 0.05 were considered significant.

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**RESULTS**

**Targeted disruption of the Gpr128 gene**

To investigate the potential roles of Gpr128 in mice, we generated a targeted disruption of the mouse Gpr128 gene in ES cells by homologous recombination. In the targeting vector, 3 exons (10, 11 and 12), which encode the GPS domain and a portion of 7TM domain, were replaced with a PGK cassette followed by the neomycin resistance gene (Figure 1A). After electroporating ES cells with the linearized targeting vector under positive-negative selection, we identified three targeted ES clones by PCR (Figure 1B). Two of these clones were microinjected into C57BL/6 blastocysts to obtain chimeras. Mice heterozygous for Gpr128 showed normal development and were fertile, indicating that the targeted locus does not have detrimental dominant activity.

The genotypes of the offspring were analyzed by PCR to identify WT (+/+), heterozygous (+/-), and homozygous (-/-) mice. Amplification of the WT and targeted alleles produced bands of 5.4 and 5.7 kb, respectively (Figure 1C). As expected, the ratio of phenotypes was in accord with Mendelian frequency, indicating that there was no increased embryonic mortality in the mutant animals. Semi-quantitative RT-PCR and immunofluorescence staining demonstrated that Gpr128 was not detected in the intestine of homozygous mice (Figure 1D and E), indicating that we have successfully established a Gpr128 disruption mouse model.

**Gpr128 is specifically expressed in the mouse intestine**

We investigated the expression pattern of the WT Gpr128 gene in adult mouse tissues by semi-quantitative RT-PCR, Northern blotting and immunofluorescence staining. Gpr128 mRNA was highly and exclusively detected in the intestine (Figure 2A, B and D). RT-PCR was then performed to determine the presence of Gpr128 mRNA throughout the digestive tract and at different postnatal development stages. Gpr128 expression was detected prominently in the small intestine and colon from postnatal day 0 through 8 wk (Figure 2C). The distribution of Gpr128 protein in the mouse intestine was then analyzed by immunofluorescence staining. We found that the Gpr128 protein was confined to the mucosa. As shown in Figure 2D, Gpr128 expression was restricted to epithelial cells.

**Gpr128<sup>−/−</sup> mice gained significantly less body weight than their WT counterparts**

Mice lacking the Gpr128 gene (Gpr128<sup>−/−</sup>) grew normally and displayed normal reproductive functions on a standard mouse chow diet. We found no differences between Gpr128<sup>−/−</sup> and Gpr128<sup>+/+</sup> mice with respect to food intake or fecal excretion (Figure 3B and C). However, Gpr128<sup>−/−</sup> mice gained less weight on average than their Gpr128<sup>+/+</sup> littermates by 24 wk of age. The body weights of WT and Gpr128<sup>−/−</sup> mice were 30.81 ± 2.84 and 25.74 ± 4.50 g, respectively (Figure 3A, n = 10, P < 0.01). When separated by sex, both male and female Gpr128<sup>−/−</sup> mice gained...
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**A** Gene targeting strategy. Numbered boxes represent Gpr128 coding exons. The start codon and stop codon are indicated as a star and pound sign, respectively. The targeting vector contains a 7.1-kb 5' arm and a 5.3-kb 3' arm. Exons 10, 11 and 12 of the Gpr128 gene were replaced by a PGK-Neo cassette through homologous recombination. The primer pairs for polymerase chain reaction (PCR) genotyping are indicated by arrows (5' arm: P1, P2; 3' arm: P3, P4); B: PCR screening for targeted embryonic stem (ES) cell clones. Correctly recombined clones show 7.7 and 5.7 kb bands, respectively. Three recombinant ES cell clones show the expected bands as detected with primers P1-P4; C: PCR analysis of genomic tail DNA derived from Gpr128+/- mouse intercrossing. A 5.4-kb fragment amplified with primers P5 and P6 represents the wild-type (WT) allele. A 5.7-kb band was amplified from the targeted allele with P3 and P4; D: Gpr128 expression in gastrointestinal tissue with two different genotypes by semiquantitive reverse transcription-polymerase chain reaction. A specific Gpr128 fragment, which exists in WT mice, was deleted in Gpr128-/- mice. The transcript for β-actin was examined as a control for RNA loading and integrity; E: Expression pattern of Gpr128 protein in WT and Gpr128-/- adult mouse colon revealed by immunofluorescence (original magnification, × 200). M: Marker lane; (-): Negative control without template; S. intestine: Small intestine; P. colon: Proximal colon; D. colon: Distal colon.

Figure 1  Targeted deletion of Gpr128 in mice. A: Gene targeting strategy. Numbered boxes represent Gpr128 coding exons. The start codon and stop codon are indicated as a star and pound sign, respectively. The targeting vector contains a 7.1-kb 5' arm and a 5.3-kb 3' arm. Exons 10, 11 and 12 of the Gpr128 gene were replaced by a PGK-Neo cassette through homologous recombination. The primer pairs for polymerase chain reaction (PCR) genotyping are indicated by arrows (5' arm: P1, P2; 3' arm: P3, P4); B: PCR screening for targeted embryonic stem (ES) cell clones. Correctly recombined clones show 7.7 and 5.7 kb bands, respectively. Three recombinant ES cell clones show the expected bands as detected with primers P1-P4; C: PCR analysis of genomic tail DNA derived from Gpr128+/- mouse intercrossing. A 5.4-kb fragment amplified with primers P5 and P6 represents the wild-type (WT) allele. A 5.7-kb band was amplified from the targeted allele with P3 and P4; D: Gpr128 expression in gastrointestinal tissue with two different genotypes by semiquantitive reverse transcription-polymerase chain reaction. A specific Gpr128 fragment, which exists in WT mice, was deleted in Gpr128-/- mice. The transcript for β-actin was examined as a control for RNA loading and integrity; E: Expression pattern of Gpr128 protein in WT and Gpr128-/- adult mouse colon revealed by immunofluorescence (original magnification, × 200). M: Marker lane; (-): Negative control without template; S. intestine: Small intestine; P. colon: Proximal colon; D. colon: Distal colon.

less weight than their WT counterparts (data not shown). The decreased weight gain in Gpr128-/- mice persisted at 28 and 32 wk (26.69 ± 4.29 and 28.46 ± 4.42 g vs 33.15 ± 3.20 and 36.75 ± 4.18 g in Gpr128+/- mice, n = 10, P < 0.01, Figure 3A).

To account for the differences in body weight gain between the Gpr128+/+ and Gpr128-/- mice, various tissues were removed and weighed. There were no differences in the epididymal and uterine fat pads, brown fat, or liver weights between male and female Gpr128+/+ and Gpr128-/- mice (Figure 3D). There were also no differences in heart, spleen, lung, and kidney weights between the Gpr128+/+ and Gpr128-/- mice (Figure 3D).

The cell counts and biochemical parameters of the
blood of Gpr128−/− mice were not different from those of the WT mice (Figure 3E and F). Furthermore, there were no overt differences in the gross morphology or histology (HE staining) of the GI tract between the Gpr128−/− and the WT mice (data not shown).

**Increased frequency of peristalsis and slow waves of the small intestine in Gpr128−/− mice**

Using a Trendelenburg model, we analyzed the peristalsis and the slow waves of the small intestine (jejunum) in WT and Gpr128−/− mice (Figure 4A). The amplitudes of peristaltic movement at resting intraluminal pressures of 0, 1, 2 and 3 cmH2O were not different between WT and Gpr128−/− mice (data not shown). The frequency of peristaltic contraction was increased in Gpr128−/− mice since 8 wk when the resting intraluminal pressure increased. The frequency of peristalsis was higher in Gpr128−/− mice than in WT mice when the resting intraluminal pressure was 3 cmH2O (6.6 ± 2.3 peristalsis/15 min in Gpr128−/− intestine vs 2.6 ± 1.7 peristalsis/15 min in WT intestine, n = 5, P < 0.05, Figure 4B). At the age of 32 wk, the frequency of peristalsis was higher in Gpr128−/− mice than in WT mice when the resting intraluminal pressure was 2 or 3 cmH2O (8.3 ± 3.0 and 7.4 ± 3.1 peristalsis/15 min in Gpr128−/− intestine vs 4.6 ± 2.3 and 3.1 ± 0.8 peristalsis/15 min in WT intestine, n = 8, 2 cmH2O: P < 0.05, 3 cmH2O: P < 0.01, Figure 4C) and the frequency of slow waves was also higher in Gpr128−/− intestine compared
with WT intestine (30.6 ± 4.2, 31.4 ± 3.9, and 31.9 ± 4.5/min and 35.8 ± 4.3, 36.4 ± 4.2, and 37.1 ± 4.8/min in normal and Gpr128^-/- mice, respectively, n = 8, P < 0.05, Figure 4D).

**DISCUSSION**

Here, we describe the first genetic analysis of Gpr128 function in a mammalian model. A targeted mutation of
A number of factors may potentially participate in the regulation of energy balance and weight gain, including gastric emptying, gastroduodenal motility as well as gastrointestinal peptides such as ghrelin and cholecystokinin. The release of these two hormones is known to be regulated by ingestion and their action may in turn regulate gastrointestinal function and food intake.

A major finding in the Gpr128<sup>−/−</sup> mice was the lower body weight gain compared with the WT littermates by 24 wk of age when the animals were maintained on a standard laboratory rodent chow diet. Additionally, there were no significant differences in the weights of epididymal or uterine fat pads, brown fat, or the liver between WT and Gpr128<sup>−/−</sup> mice. These data suggest that the observed weight difference between the mice was not due to reduced adiposity in the Gpr128 knockout mice.

GPR128 causes a deletion of part of the 7TM region (Figure 1A) and is presumably a null allele. Residual WT transcripts could not be detected in the intestines of mutant mice (Figure 1D and E).

GPR128 is an orphan GPCR, the physiological function of which is unknown. To explore the role of Gpr128, we first examined its expression profile in different tissues. We found that Gpr128 mRNA expression is exclusively confined to the small intestine and colon. Through immunofluorescence staining, Gpr128 immunoreactivity was detected in the mucosa of the intestine and was found to be restricted to epithelial cells.

The cell count and biochemical parameters of Gpr128<sup>−/−</sup> mice were not different from those of their WT counterparts, indicating that Gpr128 is not essential for the maintenance of homeostasis.

Gpr128 deficiency leads to increased frequency of intestinal contraction. A: The raw traces of intraluminal pressure of a jejunum segment of Gpr128<sup>−/−</sup> mice and the simultaneously recorded extracellular electrical potential from the gut wall. The lower panel of A shows an expanded view of the recording within the square of the upper panel; B: Frequency of peristalsis in wild-type (WT) and Gpr128<sup>−/−</sup> mice of 8 wk. The frequency of peristalsis was increased in Gpr128<sup>−/−</sup> mice at a resting intraluminal pressure of 3 cmH<sub>2</sub>O (<i>n</i> = 5, <i>P</i> = 0.0137); C: Frequency of peristalsis in WT and Gpr128<sup>−/−</sup> mice of 32 wk. The frequency of peristalsis was increased in Gpr128<sup>−/−</sup> mice at resting intraluminal pressures of 2 and 3 cmH<sub>2</sub>O (<i>n</i> = 8, 2 cmH<sub>2</sub>O: <i>P</i> = 0.0166, 3 cmH<sub>2</sub>O: <i>P</i> = 0.0020); D: Frequency of slow waves in WT and Gpr128<sup>−/−</sup> mice of 32 wk. The frequency of slow waves was increased in Gpr128<sup>−/−</sup> mice at resting intraluminal pressures of 1, 2 and 3 cmH<sub>2</sub>O (<i>n</i> = 8, 1 cmH<sub>2</sub>O: <i>P</i> = 0.0303, 2 cmH<sub>2</sub>O: <i>P</i> = 0.0271, and 3 cmH<sub>2</sub>O: <i>P</i> = 0.0402). All values are mean ± SD ("<i>P</i> < 0.05, "<i>P</i> < 0.01 vs wild-type group.")
However, given that Gpr128 and WT mice consumed equivalent amounts of chow, the excretion of feces was similar in the two groups and Gpr128 was confined to the intestinal tissue, we tested the potential differences in intestinal motility between Gpr128-/- and WT mice. The frequency of peristaltic movement and slow waves were found to be increased in Gpr128-/- intestine compared with WT intestine. Despite similar levels of chow consumption, Gpr128-/- mice colonized with the model fermentative community are significantly leaner and lighter than their WT littermates because their increased intestinal motility reduces the time required to harvest energy from the diet[3]. Whether the increase in gut motility accounts for the lower weight gain in Gpr128-/- mice awaits further investigation. Because peristalsis is known to be regulated by the enteric nerve plexus[4], whereas the slow waves are known to originate from the interstitial cells of Cajal[5], further studies should be conducted to examine their development and function in Gpr128-/- mice. Given the epithelial localization of Gpr128 within the gut, it will also be important to explore its role in the regulation of intestinal secretion and absorption.

In summary, the present study shows that Gpr128 is expressed exclusively in the small and large intestine, and Gpr128 deficiency resulted in a decrease in body weight gain and an increase in intestinal motility. The potential for Gpr128 as a novel therapeutic target for obesity and nutritional disorders is worth exploring.

REFERENCES

1 Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehozcky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Graffham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Showkenne R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendel MC, Delehaunty KD, Miller T, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson W, Wenning S, Slezak T, Doggett N, Chen JF, Olsen A, Lucas S, Elkin C, Ubbachester B, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madaan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimson J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lebrach H, Reinhardt R, McCombe WR, de la Bastide M, Dedhia N, Wallis J, Wheeler R, Hornischer K, Nisand G, Ayayarala R, Bailey JA, Bateman A, Batsoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert SG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kasprzyk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korl I, Kulp D, Lancet D, Lowe TM, McLyngsat A, Mikkelsen T, Moran JV, Muleid N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YL, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Oseegawa K, Shizuya H, Choi S, Chen YJ. Initial sequencing and analysis of the human genome. Nature 2001; 409: 866-921 [PMID: 11237011 DOI: 10.1038/33057082]

2 Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Ananatides P, Ballew RM, Huson DH, Worman JR, Zhang Q, Kodira CD, Zheng HY, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ,
Huang YS, Chang NY, Hu CH, Hsiao CC, Cheng KF, Tsai WP, Yona S, Stacey M, Gordon S, Chang GW, Lin HH. Activation of myeloid cell-specific adhesion class G protein-coupled receptor EMR2 via ligand-induced translocation and interaction with receptor subunits in lipid raft microdomains. *Mol Cell Biol* 2012; 32: 1408-1420 [PMID: 22310602 DOI: 10.1099/mcb.06557-11]

Yona S, Lin HH, Siu WO, Gordon S, Stacey M. Adhesion-GPCRs: emerging roles for novel receptors. *Trends Biochem Sci* 2008; 33: 491-500 [PMID: 18789697 DOI: 10.1016/j.tibs.2008.07.007]

Davies B, Baumann C, Kirchhoff C, Ivel R, Nubmeyer B, Habeniacht UF, Theuring F, Gottwald U. Targeted deletion of the epidymal protein HE6 results in fluid dysregulation and male infertility. *Mol Cell 2004;* 29: 8642-8648 [PMID: 15367682 DOI: 10.1038/nrd25182008]

Piao X, Hill RS, Bodell A, Chang BS, Basel-Vanagaitė L, Strausberg R, Dobyns WB, Qasrawi B, Winter RM, Innes AM, Volt T, Ross ME, Michaud JL, Désare JC, Barkovich AJ, Walsh CA. G protein-coupled receptor-dependent development of human frontal cortex. *Science* 2004; 303: 2033-2036 [PMID: 15044805 DOI: 10.1126/science.1092780]

Luo R, Jeong SJ, Jin Z, Strokes N, Li S, Piao X. G protein-coupled receptor S6 and collagen III, a receptor-ligand pair, regulates cortical development and lamination. *Proc Natl Acad Sci USA* 2011; 108: 12925-12930 [PMID: 21768377 DOI: 10.1073/pnas.1104281108]

Shashidhar S, Lorente G, Nagavarapu U, Nelson A, Kuo J, Cummins F, Nikolic K, Uffer F, Fehr ED. GPR56 is a GPCR that is overexpressed in gliomas and functions in tumor cell adhesion. *Oncoogene* 2005; 24: 1673-1682 [PMID: 15674329 DOI: 10.1089/sjc.1208395]

Ke N, Sundaram R, Liu G, Chiosis J, Fan W, Rogers C, Awdad T, Girman M, Yu D, Wong-Staal F, Li QX. Orphan G protein-coupled receptor GPR56 plays a role in cell transformation and tumorigenesis involving the cell adhesion pathway. *Mol Cancer Ther* 2007; 6: 1840-1850 [PMID: 17575113 DOI: 10.1158/1055-7163.MCT-07-0066]

Fredriksson R, Gloriam DE, Höglund PJ, Lagerström MC, Schöth OB. There exist at least 30 human G-protein-coupled receptors with long Ser/Thr-rich N-termini. *Biochem Biophys Res Commun* 2003; 301: 725-734 [PMID: 12565841 DOI: 10.1016/s0006-291x(03)0026-3]

Fredriksson R, Schöth OB. The repertoire of G-protein-coupled receptors in fully sequenced genomes. *Mol Pharm* 2005; 31: 1414-1425 [PMID: 15867224 DOI: 10.1124/molecularpharmacology.309040]

3 Alexander SP, Mathie A, Peters JA. Guide to Receptors and Channels (GRAC), 5th edition. *Br J Pharm* 2011; 164 Suppl 1: S1-324 [PMID: 22040146 DOI: 10.1111/j.1365-2179.2011.04691_1.x]

4 Rosenbaum DM, Rasmussen SG, Kobikka BK. The structure and function of G-protein-coupled receptors. *Nature* 2009; 459: 356-363 [PMID: 19458711 DOI: 10.1038/nature07844]

5 Latek D, Mądzewska A, Trzaskowski B, Palczewski K, Filipiak S. G protein-coupled receptors—recent advances. *Acta Biochim Pol* 2012; 59: 515-529 [PMID: 23251911]

6 Drex J. Drug discovery: a historical perspective. *Science* 2000; 287: 1960-1964 [PMID: 10720314 DOI: 10.1126/science.287.5460.1960]

7 Bjarnadóttir TK, Fredriksson R, Schöth OB. The adhesion GPCRs: a unique family of G-protein-coupled receptors with important roles in both central and peripheral tissues. *Cell Mol Life Sci* 2007; 64: 2104-2119 [PMID: 17502995 DOI: 10.1007/s00018-007-0706-1]

8 Lagerström MC, Schöth OB. Structural diversity of G-protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov* 2008; 7: 339-357 [PMID: 18382464 DOI: 10.1038/nrd25182008]

9 Foord SM, Jupe S, Holbrook J. Bioinformatics and type II G-protein-coupled receptors. *Biochem Soc Trans* 2002; 30: 475-479 [PMID: 12196118 DOI: 10.1042/BST0300475]
Ni YY et al. Deletion of Gpr128 in mice

24 Nagy A, Rossant J, Nagy R, Abramow-Newerly W, Roder JC. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. Proc Natl Acad Sci USA 1993; 90: 8424-8428 [PMID: 8378314 DOI: 10.1073/pnas.90.18.8424]

25 Cunningham KM, Daly J, Horowitz M, Read NW. Gastro-intestinal adaptation to diets of differing fat composition in human volunteers. Gut 1991; 32: 483-486 [PMID: 2040469 DOI: 10.1136/gut.32.5.483]

26 Boyd KA, O’Donovan DG, Doran S, Wishart J, Chapman IM, Horowitz M, Feinle C. High-fat diet effects on gut motility, hormone, and appetite responses to duodenal lipid in healthy men. Am J Physiol Gastrointest Liver Physiol 2003; 284: G188-G196 [PMID: 12409281 DOI: 10.1152/ajpgi.00375.2002]

27 Lee HM, Wang G, Englander EW, Kojima M, Greeley GH. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. Endocrinology 2002; 143: 185-190 [PMID: 11751608 DOI: 10.1210/en.143.1.185]

28 Verhulst PJ, Deportere I. Ghrelin’s second life: from appetite stimulator to glucose regulator. World J Gastroenterol 2012; 18: 3183-3195 [PMID: 22783041]

29 French SJ, Murray B, Rumsey RD, Fadzlin R, Read NW. Adaptation to high-fat diets: effects on eating behaviour and plasma cholecystokinin. Br J Nutr 1995; 73: 179-189 [PMID: 7718539 DOI: 10.1079/BJN19950022]

30 Stengel A, Taché Y. Interaction between gastric and upper small intestinal hormones in the regulation of hunger and satiety: ghrelin and cholecystokinin take the central stage. Curr Protein Pept Sci 2011; 12: 293-304 [PMID: 21428875 DOI: 10.2174/138920311795906673]

31 Samuel BS, Shaito A, Mutoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowely J, Yanagisawa M, Gordon JI. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci USA 2008; 105: 16767-16772 [PMID: 18931303 DOI: 10.1073/pnas.0808567105]

32 Costa M, Brookes SJ, Hennig GW. Anatomy and physiology of the enteric nervous system. Gut 2000; 47 Suppl 4: iv15-iv19; discussion iv26 [PMID: 11076898 DOI: 10.1136/gut.47. suppl_4.iv15]

33 Huizinga JD, Zarate N, Farrugia G. Physiology, injury, and recovery of interstitial cells of Cajal: basic and clinical science. Gastroenterology 2009; 137: 1548-1556 [PMID: 19778538 DOI: 10.1053/j.gastro.2009.09.023]

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