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Anatomy

Expression of the G protein-coupled receptor (GPR) 37 and GPR37L1 in the mouse digestive system

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ABSTRACT. G protein-coupled receptor (GPR) 37 and GPR37L1 are known to modulate the dopaminergic neuron activity, and recently, they are identified as candidate prosaposin receptors. Intercellular prosaposin is proteolytically processed into four saposins, each of which acts as a sphingolipid hydrolase activator in the lysosome. In contrast, extracellular prosaposin exerts a trophic effect on neurons via GPR37 and GPR37L1. In this study, the expression patterns of GPR37 and GPR37L1 in the mouse digestive system were examined immunohistochemically. The islets of Langerhans of the pancreas showed intense immunoreactivity for GPR37 and GPR37L1. Weak immunoreactivity for GPR37 and GPR37L1 was found in the nerve plexuses of the esophagus and small and large intestines. Colocalization of GPR37 and tyrosine hydroxylase immunoreactivity was observed in the neuron of the nerve plexus of the large intestine. This study suggests the possibility that prosaposin affects the function of islet-secreting cells. Also, the expression of GPR37 and GPR37L1 in the nerve plexus suggests that prosaposin exerts a trophic effect not only in the central nervous system, but also in the enteric nervous system.

KEY WORDS: immunohistochemistry, islet of Langerhans, neurotrophic factor, pancreas, prosaposin

Prosaposin is a glycoprotein of approximately 70 kDa molecular mass and is composed of four small domains (approximately 80 amino acid residues each)−saposins A, B, C, and D [20, 35]. In the lysosome, prosaposin is proteolytically processed into four saposins, each of which activates specific lysosomal sphingolipid hydrolases including cerebrosidase, ceramidase, sphingomyelinase, galactosidase, and arylsulfatase A [12, 21, 25], and accelerates lipid catabolism in the lysosome [4, 5, 28, 38, 41]. Therefore, cytoplasmic prosaposin plays pivotal roles in normal lysosomal function and its deficiency causes lysosomal storage diseases [33]. Also, prosaposin knock-out mice exhibit viviparous lethality or die by 35–38 days after birth with severe neurodegeneration [9].

Prosaposin is present not only in the cytoplasm but also in body fluids including serum, cerebrospinal fluid, milk, semen, bile, and pancreatic juice [14, 16, 22]. Extracellular prosaposin acts as a neurotrophic factor, which is mediated by its saposin C domain [34], and the administration of prosaposin or an artificial peptide mimicking the neurotrophic-activity region of prosaposin exerted multiple neurotrophic effects, such as rescue of hippocampal CA1 neurons from lethal ischemic damage [23, 37], prevention of structural and functional disorders of the peripheral nerves in diabetes [1, 2], and improvement of neuron survival and behavioral deficits in a model of Parkinson's disease [10]. The prosaposin receptor implicated in these neurotrophic effects is a G protein-coupled receptor (GPR) because the neurotrophic effect was suppressed by pertussis toxin [3, 15]. Among orphan GPRs, GPR 37 and GPR37L1 are considered candidate prosaposin receptors because prosaposin stimulates GPR37 and GPR37L1 signaling, and exerts a protective effect against oxidative stress in astrocytes [29, 30]. However, these receptors have also been reported to be

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unresponsive to prosaposin in HEK293 cells and yeast [6, 11, 32]. Therefore, the receptor for prosaposin remains controversial, although Liu *et al.* [27] demonstrated the protective effects of prosaposin via the GPR37/GPR37L1 pathway and provided an explanation as to why these receptors are unresponsive in HEK293 cells and yeast. Therefore, examining the relationship between the expression patterns of these receptors and the function of prosaposin in various organs may be useful to understand the prosaposin-GPR37/GPR37L1 signaling pathway.

GPR37 and GPR37 L1 are expressed abundantly in the central nervous system [24, 42]. GPR37 is insoluble and accumulates in the endoplasmic reticulum of dopaminergic neurons, leading to cell death in an autosomal recessive juvenile Parkinsonism (AR-JP), which involves a defect in ubiquitin protein ligase parkin [17, 18]; therefore, it is also known as parkin-associated endothelin-like receptor. GPR37L1 is also related to dopaminergic neuron activity, and to the endothelin (ET) receptor, although it is not activated by ET [24]. GPR37L1 and GPR37 are closely related to bombesin and several orphan G protein-coupled receptors (GPCRs), due to a similarity in the "contact-informed neighboring pocket metric" of the GPCR binding site [32]. Herts *et al*. [13] reported that, of two splice variants of the dopamine 2 receptor (D2R), GPR37 interacts more strongly with the long isoform of D2R, while GPR37L1 has a preference for the shorter D2R isoform. The expression patterns and/or the functions of these receptors in the central nervous system have attracted researchers' attention because of their associations with neural disorders and the neurotrophic activity of prosaposin. However, their expression in the digestive system is unclear although prosaposin is present in body fluids such as serum, bile, and pancreatic juice. Therefore in this study, the expression patterns of GPR37 and GPR37L1 in the mouse digestive system were examined immunohistochemically.

MATERIALS AND METHODS

Western blot

Two adult male ddY mice (35 and 40 g in weight) were obtained from Japan SLC, Inc. (Shizuoka, Japan). They were housed at 24 ± 2°C under a 12/12 hr light/dark cycle and were provided food and water *ad libitum*. Before the experiment, the mice were anesthetized by intraperitoneal injection of 8×10^{-3} ml/g body weight of Somnopentyl (Kyoritsu Seiyaku Co., Tokyo, Japan) and euthanized by decapitation. The cerebellum was dissected and homogenized in CelLytic reagent (Sigma-Aldrich Co., St. Louis, MO, USA), and the supernatant was obtained by centrifugation at 12,000 × *g* for 10 min. The protein concentration of the supernatant was determined by the Bradford assay using the Bio-Rad Protein Assay Kit II (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The protein sample was mixed 1:1 with a sample buffer solution (Nacalai Tesque Inc., Kyoto, Japan), to which β-mercaptoethanol (Nacalai Tesque) and sodium lauryl sulfate (Nacalai Tesque) were added to a final concentration of 1% each, before heating at 95°C for 5 min. After cooling on ice, the sample solution containing 20 µg of protein was subjected to 10% SDS-PAGE. The marker protein (Precision Plus Protein Standard; Bio-Rad) was loaded onto another well on the same gel according to the manufacturer's protocol. The sample was transferred to a PVDF membrane (ATTO Co., Tokyo, Japan) by semidry electroblotting at 100 mA for 60 min. The marker protein lane on the PVDF membrane was cut and stained with reversible Coomassie-blot solution containing 0.05% Coomassie Brilliant Blue R-250 (Bio-Rad), 40% methanol, and 7% acetic acid. The remaining membrane was incubated in Tris-buffered saline containing 0.05% Tween 20 (TBST; pH 7.4) supplemented with 5% nonfat dry milk and 10% blocking reagent (Roche, Mannheim, Germany) for 60 min, followed by incubation in a rabbit anti-GPR37 polyclonal antibody (bs-13534R; Bioss Antibodies, Woburn, MA, USA) diluted 1:1,000, or a rabbit anti-GPR37L1 polyclonal antibody (Bioss Antibodies, bs-15390R) diluted 1:1,000 with TBST containing 5% nonfat dry milk and 10% blocking reagent (Roche) for 60 min. After rinsing three times (10 min each) in TBST, the membrane was incubated in peroxidase-conjugated goat anti-rabbit IgG (Chemicon, Temecula, CA, USA) diluted 1:1,500 with TBST containing 5% nonfat dry milk and 10% blocking reagent (Roche) for 60 min. After another rinse in TBST, the membrane was colorized in TBST containing 0.02% 3–3′ diaminobenzidine tetrahydrochloride (DAB) and 0.003% H_2O_2.

Tissue preparation for immunohistochemistry

Six adult male ddY mice weighing 38–48 g were obtained from Japan SLC, Inc. They were housed at 24 ± 2°C under a 12/12 hr light/dark cycle and provided food and water *ad libitum*. Before the experiment, the mice were anesthetized with an intraperitoneal injection of Somnopentyl (8×10^{-3} ml/g body weight) and euthanized by cardiac perfusion with physiological saline and 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4). The esophagus, stomach, liver, pancreas, and small and large intestines were removed and immersed in the same fixative for 2–3 days. Next, they were transferred to 30% sucrose in 0.1 M PBS at 4°C for 2–3 days. After the specimen sank in the solution, it was embedded in OCT compound (Sakura Finetek Japan Co., Ltd., Tokyo, Japan), frozen at −25°C, cut transversely at 20 µm thickness on a cryostat (Microtome 5030, Bright Instruments Ltd., Bedfordshire, UK), mounted onto MAS adhesive-coated slides (Matsunami Glass Ind., Ltd., Osaka, Japan) and stored at −25°C until use.

All western blot and immunohistochemistry procedures conformed to the Regulations for Animal Experiments in Gifu University, were reviewed by the Committee for Animal Research and Welfare of Gifu University, and finally approved by the President of the University (Permission No. 17213). The Gifu University regulations conform to the Japanese Act on Welfare and Management of Animals, and Standards Relating to the Care and Keeping and Reducing Pain of Laboratory Animals (Notice of the Ministry of the Environment No. 88, 2006).

Immunohistochemistry

The sections were immersed in 0.3% H_2O_2 in methanol at room temperature for 20 min to eliminate endogenous peroxidase, followed by rinsing in PBS. For immunoperoxidase analysis, the sections were incubated with 2% normal goat serum at room temperature for 30 min. After rinsing in PBS, the sections were incubated with a rabbit anti-GPR37 polyclonal antibody (Bioss Antibodies) diluted 1:500, rabbit anti-GPR37L1 polyclonal antibody (Bioss Antibodies) diluted 1:500, or rabbit anti-PGP (protein gene product) 9.5 polyclonal antibody (Ultraclone, Wellow, UK) diluted 1:1,000 at 4°C overnight. After rinsing in PBS, the sections were incubated with biotinylated goat anti-rabbit IgG (Chemicon) diluted 1:500 at room temperature for 30 min. After rinsing in PBS, the sections were incubated with Vectastain ABC Reagent (Vector Laboratories, CA, USA) at room temperature for 30 min. Finally, the sections were colorized for 10 min in 0.1 M Tris-HCl, pH 7.4, containing 0.02% DAB and 0.003% H_2O_2, then counterstained with hematoxylin, dehydrated, and cover-slipped. For immunofluorescence, the sections were incubated with 2% normal donkey serum at room temperature for 30 min. After rinsing in PBS, the sections used to examine colocalization of GPR37 or GPR37L1 and PGP9.5 were incubated with a rabbit anti-GPR37 polyclonal antibody (Bioss Antibodies) diluted 1:500 or a rabbit anti-GPR37L1 polyclonal antibody (Bioss Antibodies) diluted 1:500 and a guinea pig anti-PGP9.5 polyclonal antibody (GeneTex Inc., Irvine, CA, USA) diluted 1:1,000 at 4°C overnight. The sections used to examine the colocalization of GPR37 and tyrosine hydroxylase (TH) were incubated with a rabbit anti-GPR37 polyclonal antibody (Bioss Antibodies) diluted 1:500, and a sheep anti-TH polyclonal antibody (Abcam, Cambridge, UK) diluted 1:1,000 at 4°C overnight. After rinsing in PBS, the sections for GPR37/GPR37L1 and PGP9.5 were incubated with a FITC-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) diluted 1:100 and a Alexa Fluor 594-conjugated donkey anti-guinea pig IgG (Jackson ImmunoResearch Laboratories) diluted 1:100, and the sections for GPR37 and TH were incubated with a AMCA-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories) diluted 1:100, and a FITC-conjugated donkey anti-sheep IgG (Jackson ImmunoResearch Laboratories) diluted 1:100 at room temperature for 90 min. Finally, some sections were rinsed in PBS, counterstained with DAPI (Dojindo Laboratories, Kumamoto, Japan) diluted 1:1,000, rinsed in PBS again, cover-slipped, and observed using the EVOS FL Cell Imaging System (Thermo Fisher Scientific). Negative controls were created by replacing the primary antibody with normal rabbit IgG (Fujifilm Wako Pure Chemical Co., Osaka, Japan). No specific staining was observed in the control sections.

RESULTS

Antibody specificity

The specificity of the GPR37 and GPR37L1 antibodies used in this study was examined in the mice cerebellum by western blotting. A single immunoreactive band, similar to the report by Li *et al.* [26], was observed for each antibody (Fig. 1); therefore, these antibodies were used for further experiments.

Loalization of GPR37 and GPR37L1 immunoreactivity in the mouse digestive system

GPR37 and GPR37L1 immunoreactivity was observed in the pancreas, esophagus, and small and large intestines. These immunoreactivities could not be observed in the stomach and liver in this study.

Pancreas

In the pancreas, the islets of Langerhans showed immunoreactivity for GPR37 (Fig. 2A and 2E) and GPR37L1 (Fig. 2B and 2F). The islets were stained intensely and uniformly, with slightly more intense staining around their peripheral edge (Fig. 2E, 2F, 2H and 2K). The uniform staining pattern of the islets indicates the expression of GPR37 and GPR37L1 by the islet secretory cells. The staining around the peripheral edge of the islets resulted from GPR37 and GPR37L1 expression in the autonomic nerve fibers penetrating the islets of Langerhans, because that region also showed intense immunoreactivity for a neuronal marker, PGP 9.5 (Fig. 2C, 2G, 2I, 2J, 2L and 2M). The pancreatic acinar cells showed no immunoreactivity for GPR37, GPR37L1, or PGP 9.5. Use of normal rabbit IgG instead of the first antibody yielded no immunoreactivity in the pancreas (Fig. 2D).

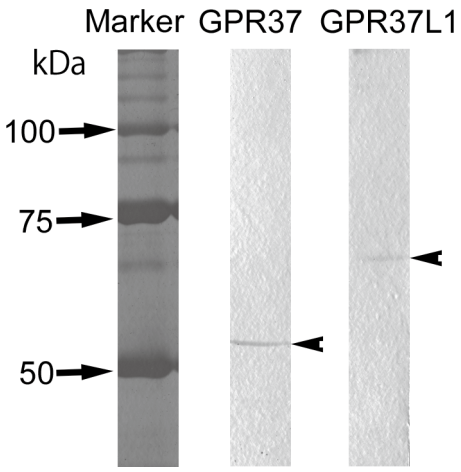

Fig. 1. Western blot of the total protein extracted from the mouse cerebellum and stained with a G protein-coupled receptor (GPR) 37 or GPR37L1 antibody. Arrowheads indicate the immunoreactive bands. The marker lane stained with Coomassie Brilliant Blue is also shown.

The digestive tract

In the esophagus, the myenteric nerve plexus showed weak immunoreactivity for GPR37 and GPR37L1 (Fig. 3A and 3B) and intense immunoreactivity for PGP 9.5 (Fig. 3C). Use of normal rabbit IgG instead of the first antibody yielded no immunoreactivity (Fig. 3D). In the small intestine, weak immunoreactivity for GPR37 and GPR37L1 was observed in the submucosal and myenteric nerve plexus (Fig. 4A and 4B). The number of nerve plexuses showing immunoreactivity for GPR37 and GPR37L1 was smaller than that showing intense immunoreactivity for PGP 9.5 (Fig. 4C). Use of normal rabbit IgG instead of the first antibody yielded no immunoreactivity (Fig. 4D). In the large intestine, the immunoreactivity of GPR37, GPR37L1, and PGP 9.5 was similar to that in the small intestine, that is,

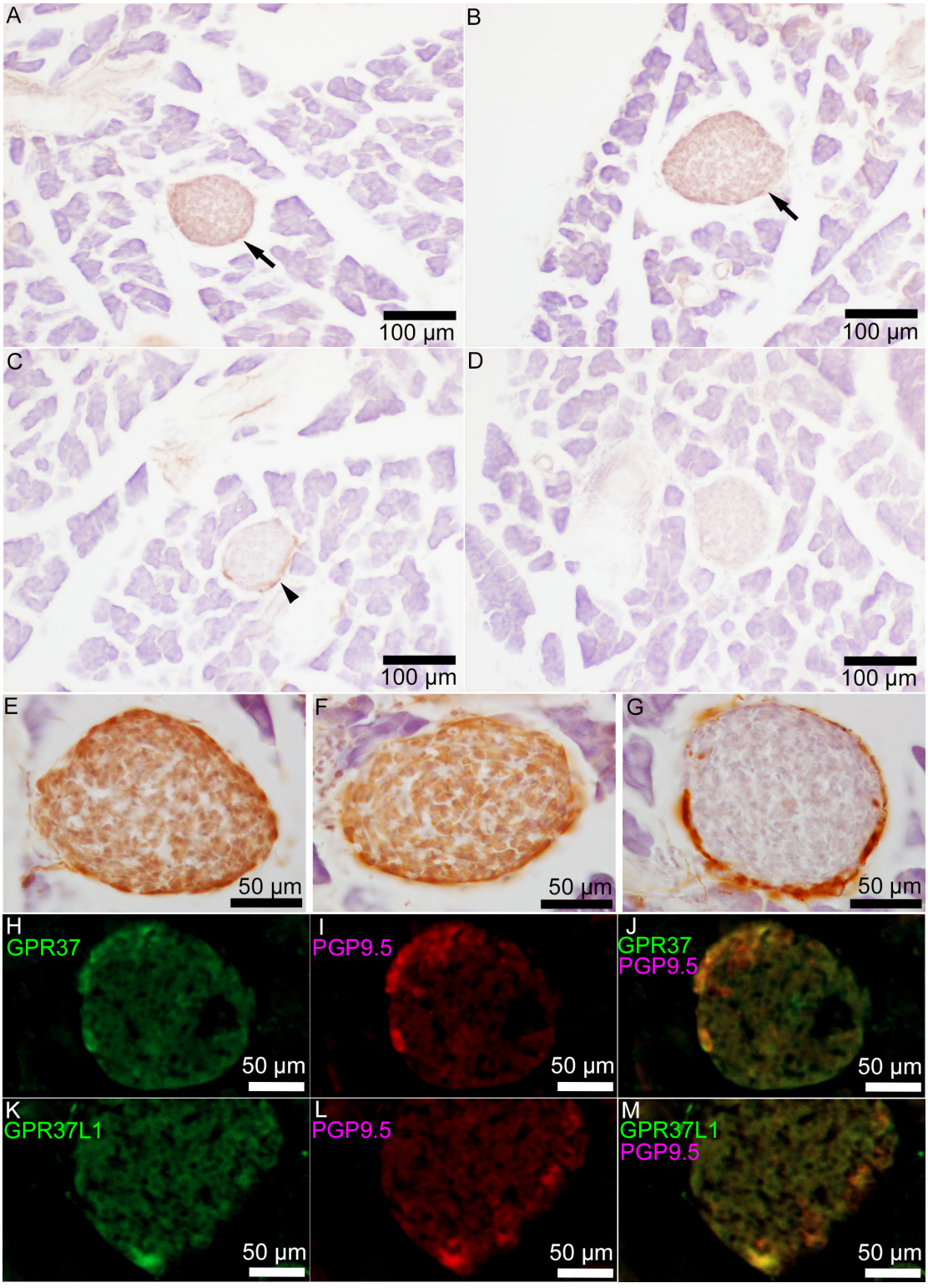

Fig. 2. Immunoreactivity in the pancreas with hematoxylin counterstaining. (A) G protein-coupled receptor (GPR) 37 immunoreactivity was found in the islets of Langerhans (arrow). The islet was stained uniformly, but the exocrine acinar cells showed no immunoreactivity. (B) GPR37L1 immunoreactivity was found in the islets of Langerhans (arrow), and its staining pattern in the pancreas was identical to that of GPR37. (C) Protein gene product (PGP) 9.5 immunoreactivity at the autonomic nerve ending was found at the peripheral edge of the islets of Langerhans (arrowhead), while the inside of the islet as well as the exocrine acinar cells showed no immunoreactivity. (D) Replacement of the first antibody with normal rabbit IgG yielded no immunoreactivity. (E–G) High magnification of islets stained with antibodies for GPR37 (E), GPR37L1 (F), and PGP 9.5 (G). (H–J) Double immunofluorescence staining of islets with the antibody for GPR37 (H) and PGP 9.5 (I), and a merged image (J). (K–M) Double immunofluorescence staining of islets with antibodies for GPR37L1 (K) and PGP 9.5 (L) and a merged image (M) GPR37/GPR37L1 immunoreactivity was detected in PGP9.5-immunopositive fibers. Bars, 100 μm in (A–D), 50 μm in (E–M).

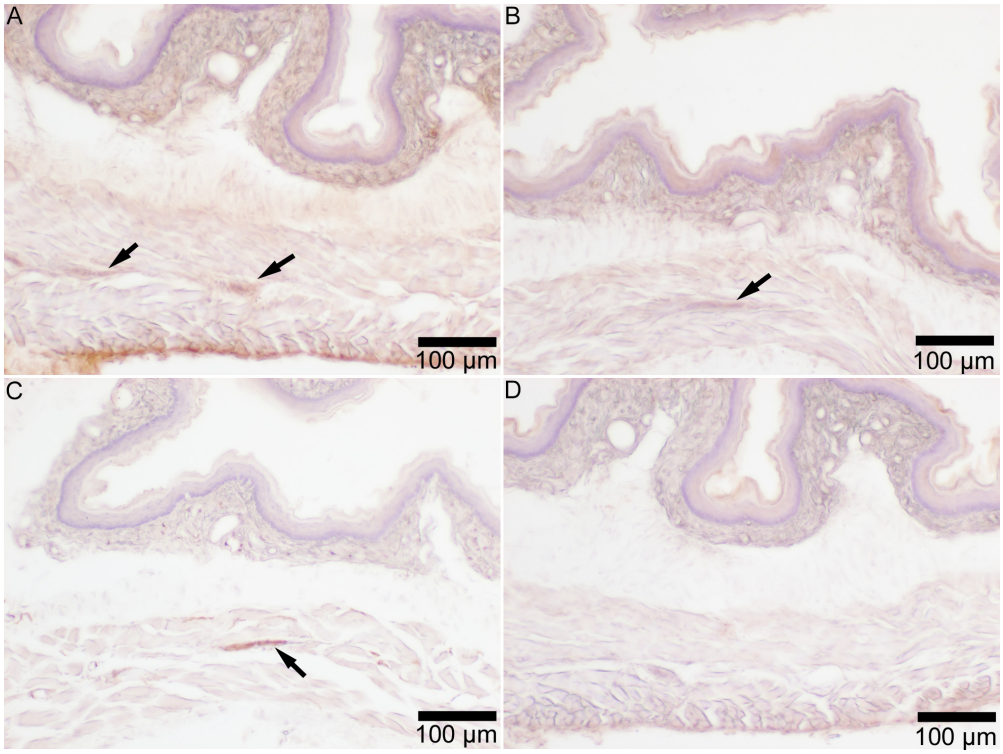

Fig. 3. Immunoreactivity in the esophagus with hematoxylin counterstaining. (A) G protein-coupled receptor (GPR) 37 immunoreactivity of weak intensity was found in the myenteric nerve plexus (arrows). (B) GPR37L1 immunoreactivity was found in the myenteric nerve plexus (arrow) with a similar staining pattern and intensity to GPR37. (C) Protein gene product (PGP) 9.5 immunoreactivity was found in the nerve plexus (arrow) as well as nerve fibers in the muscle layer. (D) Replacement of the first antibody with normal rabbit IgG yielded no immunoreactivity. Bar, 100 μm.

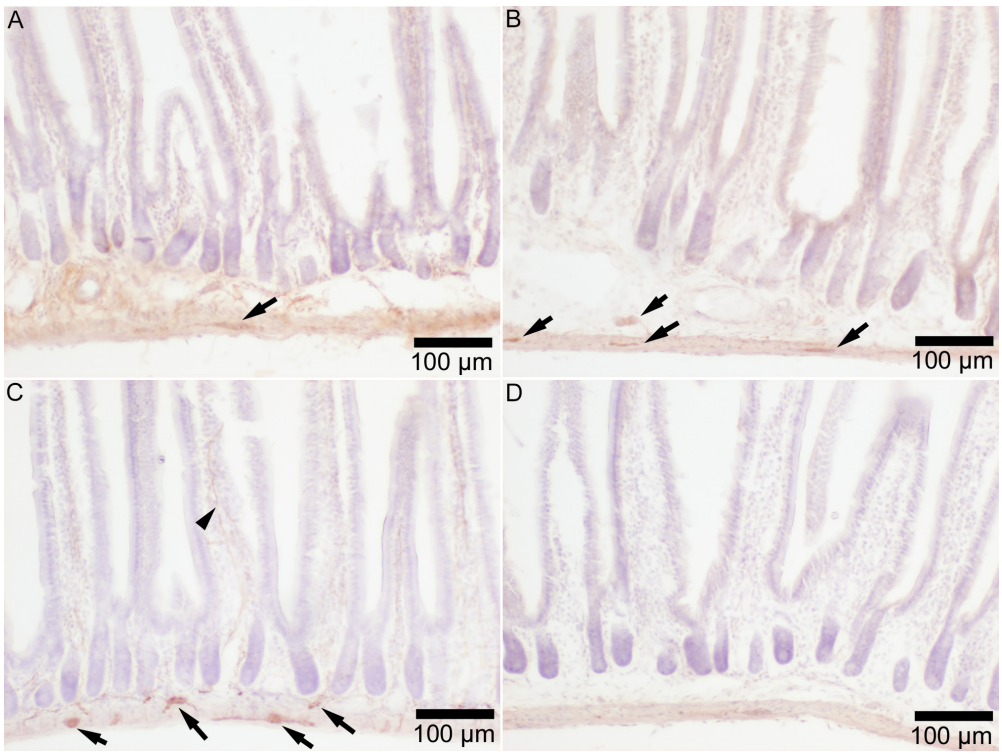

Fig. 4. Immunoreactivity in the small intestine with hematoxylin counterstaining. (A) G protein-coupled receptor (GPR) 37 immunoreactivity of weak intensity was found in the submucosal and myenteric nerve plexus (arrow). (B) GPR37L1 immunoreactivity was found in the submucosal and myenteric nerve plexus (arrows) with a similar staining pattern and intensity to GPR37. (C) Protein gene product (PGP) 9.5 immunoreactivity was found in the nerve plexus (arrows) as well as nerve fibers (arrowhead) in the submucosa and muscle layer. (D) Replacement of the first antibody with normal rabbit IgG showed no immunoreactivity. Bar, 100 μm.

some plexuses showed weak immunoreactivity for GPR37 and GPR37L1 and many showed intense immunoreactivity for PGP 9.5 (data not shown).

To confirm the localization of GPR37 and GPR37L1 in the nerve plexus, double immunofluorescence staining of the large intestine was performed. The nerve plexus as well as the surrounding fine fibers showed intense immunoreactivity for PGP 9.5 (Fig. 5A), and weak immunoreactivity was observed in the nerve plexus for GPR37 or GPR37L1 (Fig. 5B); overlapping immunoreactivity of PGP 9.5 and GPR37 or GPR37L1 was observed in the nerve plexus (Fig. 5C). Further examination concerning the colocalization of GPR37 and TH in the large intestine was performed because of the strong relationship between the receptor and dopaminergic neurons in the central nervous system. The neurons in the myenteric nerve plexus showed weak immunoreactivity for GPR37 (Fig. 6A), and a small number of them showed intense immunoreactivity for TH (Fig. 6B and 6C).

DISCUSSION

GPR37 and GPR37L1 are present not only in a variety of neurons but also in glial cells in the central nervous system [24, 42]. Also, mRNA of GPR37 and GPR37L1 has been detected by quantitative real-time polymerase chain reaction (qPCR) in the muscle layer of mouse duodenum and small and large intestines [19]. In addition, by qPCR and immunoblotting, the presence of GPR37L1 was detected in mouse heart, kidney, liver, lung, spleen, stomach, pancreas, skeletal muscle, testis, and ovary, and that GPR37L1 has been shown to participate in renal proximal tubule luminal sodium transport [43]. These findings indicate that these putative prosaposin receptors play an as-yet unknown role in various tissues. In this study, the presence of GPR37 and GPR37L1 was observed in the islets of Langerhans in the mouse pancreas. Islet-secreting cells may express these receptors to detect and secrete appropriate amounts of prosaposin in plasma, because plasma contains a considerable amount of prosaposin [36]. Most cells endowed with these receptors were possible to be insulin-secreting beta cells, because in mice, the ratio of beta cells in the islets is higher (60–80%) than that in human (50–70%). Also, the beta cells comprise a large core in mouse islets [7], and GPR37 and GPR37L1 immunoreactivity was observed almost uniformly in the islets. However, we cannot rule out the possibility that GPR37 and GPR37L1 are expressed not only by beta cells, but also by alpha, delta, and pancreatic polypeptide cells in the islets of Langerhans. Further studies, for example involving multiple immunostaining analysis of these receptors plus insulin, glucagon, somatostatin, and pancreatic polypeptide, are required to confirm our hypothesis. An examination of prosaposin expression in the islets of Langerhans is also required.

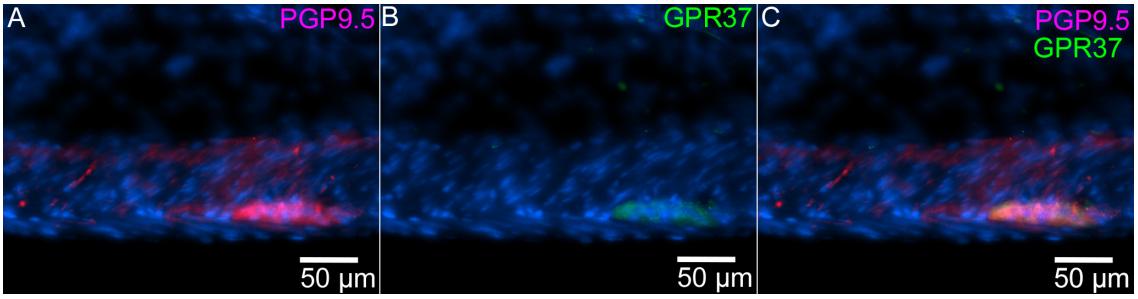

Fig. 5. Double immunofluorescence staining of the large intestine with DAPI counterstaining (blue). (A) Protein gene product (PGP) 9.5 immunoreactivity was found in the myenteric nerve plexus as well as the nerve fibers in the muscle layer (red). (B) G protein-coupled receptor (GPR) 37 immunoreactivity was found in the myenteric nerve plexus (green). (C) GPR37 immunoreactivity was detected in the PGP9.5-immunopositive cells (light yellow). Bar, 50 µm.

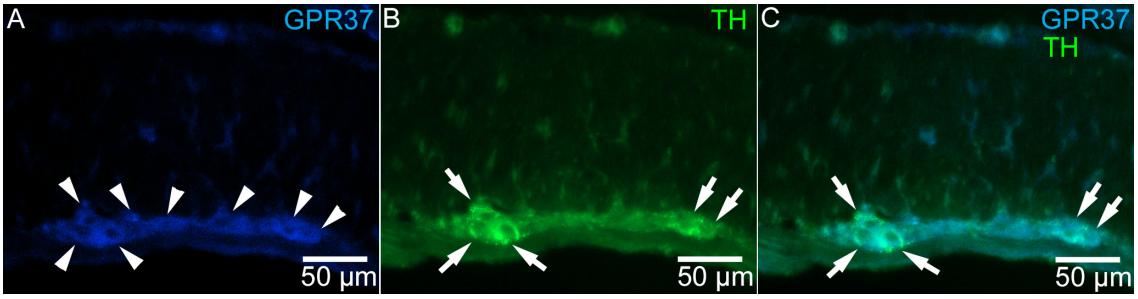

Fig. 6. Double immunofluorescence staining of the large intestine. (A) G protein-coupled receptor (GPR) 37 immunoreactivity in the myenteric nerve plexus. Arrowheads indicate GPR37 immunoreactive neurons in the plexus. (B) Tyrosine hydroxylase (TH) immunoreactivity in the same plexus. Arrows indicate TH immunoreactive neurons in the plexus. (C) Colocalization of GPR37 and TH was observed in only a small number of neurons (arrows). Bar, 50 µm.

For the digestive tract, GPR37 and GPR37L1 immunoreactivity was found in the nerve plexuses of the esophagus and small and large intestines, as determined by qPCR by Ito *et al*. [19]. However, the GPR37 or GPR37L1 immunoreactivities were weak and difficult to observe in some small plexuses. The weak immunoreactivity may have hampered visualization of immunostaining in the small plexus. It is uncertain whether all plexuses showed weak immunoreactivity. Some immunoreactive neurons also showed immunoreactivity for TH, a rate-limiting enzyme in catecholamine synthesis and thus a marker for dopaminergic and adrenergic neurons [31]. Singaram *et al*. [39] examined the distribution of dopaminergic neurons in the nerve plexus of the colon in healthy, constipated, and Parkinson's disease patients. The percentage of dopaminergic neurons in the normal colon was less than 20%, and the value decreased significantly in Parkinson's disease patients. The colocalization of GPR37/GPR37L1 and TH suggests that administration of prosaposin would attenuate gastrointestinal dysfunction in Parkinson's disease patients [8], because of the protective effect of prosaposin on dopaminergic neurons in the central nervous system [10].

Finally, GPR37 and GPR37L1 immunoreactivity was not detected in the absorptive epithelium of the gastrointestinal epithelium. Therefore, it is unlikely that prosaposin in gastrointestinal juice might stimulates the epithelial cells. The saposin domains in prosaposin facilitate the interaction between the membrane-bound lipid substrate and the water-soluble enzyme [38]; therefore, prosaposin in the gastrointestinal juice may assist digestion in the luminal space. However, it is still unknown if prosaposin is present in the digestive tract, although Sun *et al.* [40] reported its expression in mucosal epithelial cells. Further examination of prosaposin expression in the digestive system is required to elucidate the prosaposin-GPR37/GPR37L1 signaling pathway in this system.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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REFERENCES

1. Calcutt, N. A., Campana, W. M., Eskeland, N. L., Mohiuddin, L., Dines, K. C., Mizisin, A. P. and O'Brien, J. S. 1999. Prosaposin gene expression and the efficacy of a prosaposin-derived peptide in preventing structural and functional disorders of peripheral nerve in diabetic rats. *J. Neuropathol. Exp. Neurol.* **58**: 628–636. [Medline] [CrossRef]
2. Calcutt, N. A., Freshwater, J. D. and O'Brien, J. S. 2000. Protection of sensory function and antihyperalgesic properties of a prosaposin-derived peptide in diabetic rats. *Anesthesiology* **93**: 1271–1278. [Medline] [CrossRef]
3. Campana, W. M., Hiraiwa, M. and O'Brien, J. S. 1998. Prosaptide activates the MAPK pathway by a G-protein-dependent mechanism essential for enhanced sulfatide synthesis by Schwann cells. *FASEB J.* **12**: 307–314. [Medline]
4. Ciaffoni, F., Tatti, M., Salvioli, R. and Vaccaro, A. M. 2003. Interaction of saposin D with membranes: effect of anionic phospholipids and sphingolipids. *Biochem. J.* **373**: 785–792. [Medline] [CrossRef]
5. Ciaffoni, F., Tatti, M., Boe, A., Salvioli, R., Fluharty, A., Sonnino, S. and Vaccaro, A. M. 2006. Saposin B binds and transfers phospholipids. *J. Lipid Res.* **47**: 1045–1053. [Medline] [CrossRef]
6. Coleman, J. L. J., Ngo, T., Schmidt, J., Mrad, N., Liew, C. K., Jones, N. M., Graham, R. M. and Smith, N. J. 2016. Metalloprotease cleavage of the N terminus of the orphan G protein-coupled receptor GPR37L1 reduces its constitutive activity. *Sci. Signal.* **9**: ra36. [Medline] [CrossRef]
7. Dolenšek, J., Rupnik, M. S. and Stožer, A. 2015. Structural similarities and differences between the human and the mouse pancreas. *Islets* **7**: e1024405. [Medline] [CrossRef]
8. Edwards, L. L., Quigley, E. M. and Pfeiffer, R. F. 1992. Gastrointestinal dysfunction in Parkinson's disease: frequency and pathophysiology. *Neurology* **42**: 726–732. [Medline] [CrossRef]
9. Fujita, N., Suzuki, K., Vanier, M. T., Popko, B., Maeda, N., Klein, A., Henseler, M., Sandhoff, K., Nakayasu, H. and Suzuki, K. 1996. Targeted disruption of the mouse sphingolipid activator protein gene: a complex phenotype, including severe leukodystrophy and wide-spread storage of multiple sphingolipids. *Hum. Mol. Genet.* **5**: 711–725. [Medline] [CrossRef]
10. Gao, H. L., Li, C., Nabeka, H., Shimokawa, T., Saito, S., Wang, Z. Y., Cao, Y. M. and Matsuda, S. 2013. Attenuation of MPTP/MPP(+) toxicity *in vivo* and *in vitro* by an 18-mer peptide derived from prosaposin. *Neuroscience* **236**: 373–393. [Medline] [CrossRef]
11. Giddens, M. M., Wong, J. C., Schroeder, J. P., Farrow, E. G., Smith, B. M., Owino, S., Soden, S. E., Meyer, R. C., Saunders, C., LePichon, J. B., Weinshenker, D., Escayg, A. and Hall, R. A. 2017. GPR37L1 modulates seizure susceptibility: Evidence from mouse studies and analyses of a human GPR37L1 variant. *Neurobiol. Dis.* **106**: 181–190. [Medline] [CrossRef]
12. Harzer, K., Paton, B. C., Christomanou, H., Chatelut, M., Levade, T., Hiraiwa, M. and O'Brien, J. S. 1997. Saposins (sap) A and C activate the degradation of galactosylceramide in living cells. *FEBS Lett.* **417**: 270–274. [Medline] [CrossRef]
13. Hertz, E., Terenius, L., Vukojević, V. and Svenningsson, P. 2019. GPR37 and GPR37L1 differently interact with dopamine 2 receptors in live cells. *Neuropharmacology* **152**: 51–57. [Medline] [CrossRef]
14. Hineno, T., Sano, A., Kondoh, K., Ueno, S., Kakimoto, Y. and Yoshida, K. 1991. Secretion of sphingolipid hydrolase activator precursor, prosaposin. *Biochem. Biophys. Res. Commun.* **176**: 668–674. [Medline] [CrossRef]
15. Hiraiwa, M., Campana, W. M., Martin, B. M. and O'Brien, J. S. 1997. Prosaposin receptor: evidence for a G-protein-associated receptor. *Biochem. Biophys. Res. Commun.* **240**: 415–418. [Medline] [CrossRef]
16. Hiraiwa, M., O'Brien, J. S., Kishimoto, Y., Galdzicka, M., Fluharty, A. L., Ginns, E. I. and Martin, B. M. 1993. Isolation, characterization, and proteolysis of human prosaposin, the precursor of saposins (sphingolipid activator proteins). *Arch. Biochem. Biophys.* **304**: 110–116. [Medline]

[CrossRef]
17. Imai, Y., Inoue, H., Kataoka, A., Hua-Qin, W., Masuda, M., Ikeda, T., Tsukita, K., Soda, M., Kodama, T., Fuwa, T., Honda, Y., Kaneko, S., Matsumoto, S., Wakamatsu, K., Ito, S., Miura, M., Aosaki, T., Itohara, S. and Takahashi, R. 2007. Pael receptor is involved in dopamine metabolism in the nigrostriatal system. *Neurosci. Res.* **59**: 413–425. [Medline] [CrossRef]
18. Imai, Y., Soda, M., Inoue, H., Hattori, N., Mizuno, Y. and Takahashi, R. 2001. An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. *Cell* **105**: 891–902. [Medline] [CrossRef]
19. Ito, J., Ito, M., Nambu, H., Fujikawa, T., Tanaka, K., Iwaasa, H. and Tokita, S. 2009. Anatomical and histological profiling of orphan G-protein-coupled receptor expression in gastrointestinal tract of C57BL/6J mice. *Cell Tissue Res.* **338**: 257–269. [Medline] [CrossRef]
20. Kishimoto, Y., Hiraiwa, M. and O'Brien, J. S. 1992. Saposins: structure, function, distribution, and molecular genetics. *J. Lipid Res.* **33**: 1255–1267. [Medline]
21. Klein, A., Henseler, M., Klein, C., Suzuki, K., Harzer, K. and Sandhoff, K. 1994. Sphingolipid activator protein D (sap-D) stimulates the lysosomal degradation of ceramide *in vivo*. *Biochem. Biophys. Res. Commun.* **200**: 1440–1448. [Medline] [CrossRef]
22. Koochekpour, S., Hu, S., Vellasco-Gonzalez, C., Bernardo, R., Azabdaftari, G., Zhu, G., Zhau, H. E., Chung, L. W. and Vessella, R. L. 2012. Serum prosaposin levels are increased in patients with advanced prostate cancer. *Prostate* **72**: 253–269. [Medline] [CrossRef]
23. Kotani, Y., Matsuda, S., Wen, T. C., Sakanaka, M., Tanaka, J., Maeda, N., Kondoh, K., Ueno, S. and Sano, A. 1996. A hydrophilic peptide comprising 18 amino acid residues of the prosaposin sequence has neurotrophic activity *in vitro* and *in vivo*. *J. Neurochem.* **66**: 2197–2200. [Medline] [CrossRef]
24. Leng, N., Gu, G., Simerly, R. B. and Spindel, E. R. 1999. Molecular cloning and characterization of two putative G protein-coupled receptors which are highly expressed in the central nervous system. *Brain Res. Mol. Brain Res.* **69**: 73–83. [Medline] [CrossRef]
25. Li, S. C., Kihara, H., Serizawa, S., Li, Y. T., Fluharty, A. L., Mayes, J. S. and Shapiro, L. J. 1985. Activator protein required for the enzymatic hydrolysis of cerebroside sulfate. Deficiency in urine of patients affected with cerebroside sulfatase activator deficiency and identity with activators for the enzymatic hydrolysis of GM1 ganglioside and globotriaosylceramide. *J. Biol. Chem.* **260**: 1867–1871. [Medline]
26. Li, X., Nabeka, H., Saito, S., Shimokawa, T., Khan, M. S. I., Yamamiya, K., Shan, F., Gao, H., Li, C. and Matsuda, S. 2017. Expression of prosaposin and its receptors in the rat cerebellum after kainic acid injection. *IBRO Rep.* **2**: 31–40. [Medline] [CrossRef]
27. Liu, B., Mosienko, V., Vaccari Cardoso, B., Prokudina, D., Huentelman, M., Teschemacher, A. G. and Kasparov, S. 2018. Glio- and neuro-protection by prosaposin is mediated by orphan G-protein coupled receptors GPR37L1 and GPR37. *Glia* **66**: 2414–2426. [Medline] [CrossRef]
28. Locatelli-Hoops, S., Remmel, N., Klingenstein, R., Breiden, B., Rossocha, M., Schoeniger, M., Koenigs, C., Saenger, W. and Sandhoff, K. 2006. Saposin A mobilizes lipids from low cholesterol and high bis(monoacylglycerol)phosphate-containing membranes: patient variant Saposin A lacks lipid extraction capacity. *J. Biol. Chem.* **281**: 32451–32460. [Medline] [CrossRef]
29. Meyer, R. C., Giddens, M. M., Coleman, B. M. and Hall, R. A. 2014. The protective role of prosaposin and its receptors in the nervous system. *Brain Res.* **1585**: 1–12. [Medline] [CrossRef]
30. Meyer, R. C., Giddens, M. M., Schaefer, S. A. and Hall, R. A. 2013. GPR37 and GPR37L1 are receptors for the neuroprotective and glioprotective factors prosaptide and prosaposin. *Proc. Natl. Acad. Sci. USA* **110**: 9529–9534. [Medline] [CrossRef]
31. Neuhuber, W. and Wörl, J. 2018. Monoamines in the enteric nervous system. *Histochem. Cell Biol.* **150**: 703–709. [Medline] [CrossRef]
32. Ngo, T., Ilatovskiy, A. V., Stewart, A. G., Coleman, J. L. J., McRobb, F. M., Riek, R. P., Graham, R. M., Abagyan, R., Kufareva, I. and Smith, N. J. 2017. Orphan receptor ligand discovery by pickpocketing pharmacological neighbors. *Nat. Chem. Biol.* **13**: 235–242. [Medline] [CrossRef]
33. O'Brien, J. S. and Kishimoto, Y. 1991. Saposin proteins: structure, function, and role in human lysosomal storage disorders. *FASEB J.* **5**: 301–308. [Medline] [CrossRef]
34. O'Brien, J. S., Carson, G. S., Seo, H. C., Hiraiwa, M. and Kishimoto, Y. 1994. Identification of prosaposin as a neurotrophic factor. *Proc. Natl. Acad. Sci. USA* **91**: 9593–9596. [Medline] [CrossRef]
35. O'Brien, J. S., Kretz, K. A., Dewji, N., Wenger, D. A., Esch, F. and Fluharty, A. L. 1988. Coding of two sphingolipid activator proteins (SAP-1 and SAP-2) by same genetic locus. *Science* **241**: 1098–1101. [Medline] [CrossRef]
36. Sano, A., Hineno, T., Mizuno, T., Kondoh, K., Ueno, S., Kakimoto, Y. and Inui, K. 1989. Sphingolipid hydrolase activator proteins and their precursors. *Biochem. Biophys. Res. Commun.* **165**: 1191–1197. [Medline] [CrossRef]
37. Sano, A., Matsuda, S., Wen, T. C., Kotani, Y., Kondoh, K., Ueno, S., Kakimoto, Y., Yoshimura, H. and Sakanaka, M. 1994. Protection by prosaposin against ischemia-induced learning disability and neuronal loss. *Biochem. Biophys. Res. Commun.* **204**: 994–1000. [Medline] [CrossRef]
38. Schulze, H., Kolter, T. and Sandhoff, K. 2009. Principles of lysosomal membrane degradation: Cellular topology and biochemistry of lysosomal lipid degradation. *Biochim. Biophys. Acta* **1793**: 674–683. [Medline] [CrossRef]
39. Singaram, C., Ashraf, W., Gaumnitz, E. A., Torbey, C., Sengupta, A., Pfeiffer, R. and Quigley, E. M. M. 1995. Dopaminergic defect of enteric nervous system in Parkinson's disease patients with chronic constipation. *Lancet* **346**: 861–864. [Medline] [CrossRef]
40. Sun, Y., Witte, D. P. and Grabowski, G. A. 1994. Developmental and tissue-specific expression of prosaposin mRNA in murine tissues. *Am. J. Pathol.* **145**: 1390–1398. [Medline]
41. Vaccaro, A. M., Tatti, M., Ciaffoni, F., Salvioli, R., Maras, B. and Barca, A. 1993. Function of saposin C in the reconstitution of glucosylceramidase by phosphatidylserine liposomes. *FEBS Lett.* **336**: 159–162. [Medline] [CrossRef]
42. Valdenaire, O., Giller, T., Breu, V., Ardati, A., Schweizer, A. and Richards, J. G. 1998. A new family of orphan G protein-coupled receptors predominantly expressed in the brain. *FEBS Lett.* **424**: 193–196. [Medline] [CrossRef]
43. Zheng, X., Asico, L. D., Ma, X. and Konkalmatt, P. R. 2019. G protein-coupled receptor 37L1 regulates renal sodium transport and blood pressure. *Am. J. Physiol. Renal Physiol.* **316**: F506–F516. [Medline] [CrossRef]