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GPR119 expression in normal human tissues and islet cell tumors: evidence for its islet-gastrointestinal distribution, expression in pancreatic beta and alpha cells, and involvement in islet function

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Conflict of Interest

The authors have no conflict of interest to declare.

Abstract

Objective GPR119 is reportedly involved in regulating glucose metabolism and food intake in rodents, but little is known about its expression and functional significance in humans. To begin to assess the potential clinical importance of GPR119, was examined the distribution of *GPR119* gene expression in humans. ***Materials/Methods*** Expression of GPR119 mRNA in fresh samples of normal human pancreas (*n*=19) and pancreatic islets (*n*=3) and in insulinomas (*n*=2) and glucagonomas (*n*=2), all collected at surgery, were compared with the mRNA expression of various receptors highly expressed and operative in human pancreatic islets. ***Results*** GPR119 mRNA was most abundant in the pancreas, followed by the duodenum, stomach, jejunum, ileum and colon. Pancreatic levels of GPR119 mRNA were similar to those of GPR40 mRNA and were higher than those of GLP1R and SUR1 mRNA, which are strongly expressed in human pancreatic islets. Moreover, levels of GPR119 mRNA in pancreatic islets were more than 10 times higher than in adjacent pancreatic tissue, as were levels of *GPR40* mRNA. GPR119 mRNA was also abundant in two cases of insulinoma and two cases of glucagonoma, but was undetectable in a pancreatic acinar cell tumor. Similar results were obtained with mouse pancreatic islets, MIN6 insulinoma cells and alpha-TC glucagonoma cells. ***Conclusions*** The results provide evidence of an islet-gastrointestinal distribution of GPR119, its expression in pancreatic beta and alpha cells, and its possible

involvement in islet function. They also provide the basis for a better understanding of the potential clinical importance of GPR119.

Keywords insulinoma, glucagonoma, insulin secretion, gastrointestinal hormones

Abbreviations FFPE: formalin-fixed, paraffin-embedded. GLP1R: glucagon-like peptide 1 receptor. GPR119: G protein-coupled receptor 119. GPR40: G protein-coupled receptor 40. SUR1: sulfonylurea receptor 1

1. Introduction

Endogenous lipids such as free fatty acids and acylethanolamides are known to regulate glucose metabolism and food intake [1-3]. The underlying molecular mechanisms are not fully understood, however. Recently, four orphan G protein-coupled receptors (GPR40, GPR41, GPR43 and GPR120) were deorphaned and identified as fatty acid receptors [4-8]. Among those, we found that GPR40 is highly expressed in human pancreatic beta cells and is involved in regulating insulin secretion [9, 10]. In addition, GPR119 has been identified as a Gs-coupled receptor whose putative endogenous ligands include oleoylethanolamide (OEA) [11, 12] and possibly other lipids [13-16]. *In vitro* studies have implicated GPR119 in the regulation of insulin and incretin secretion [12, 14, 15, 17-20], and *in vivo* studies in rats and mice suggest its involvement in the regulation of glucose metabolism and feeding [11, 14, 18, 19, 21-30]. That said, glucose metabolism in humans and mice may differ [31], and little is known about the expression and physiological significance of GPR119 in humans.

In that context, we examined *GPR119* gene expression in various human tissues, including fresh samples of pancreas and digestive tract collected at surgery. In addition, to gain further insight into the localization of GPR119 within the human pancreas, we compared *GPR119* expression in human pancreatic islets and adjacent pancreatic tissue, as well as in insulinomas and glucagonomas, two very rare human tumors that possess the endocrine properties of pancreatic beta and alpha cells, respectively. The results provide evidence of the

84 islet-gastrointestinal distribution of GPR119, its expression in pancreatic beta and alpha cells,

85 and its possible involvement in islet function in humans.

2. Methods

2.1. Subjects, tissue sampling and pancreatic islet isolation

The clinical profiles of all patients enrolled in the present study are shown in Table 1. The study was performed in accordance with the Declaration of Helsinki and approved by the Ethical Committee on Human Research of Kyoto University Graduate School of Medicine. Signed informed consent was obtained from all patients.

Normal human cerebral tissues (*n*=3) were collected from three patients at autopsy; one had died from amyotrophic lateral sclerosis, one from an iliopsoas muscle tumor and one from a ruptured aortic aneurysm. Normal tissues from the pancreas (*n*=19), esophagus (*n*=3), stomach (*n*=3), duodenum (*n*=3), jejunum (*n*=3), ileum (*n*=2), colon (*n*=3) and liver (*n*=2) were collected from 23 patients at tumor resection. In Fig. 1b and c, pancreatic tissues from four patients (patients 9, 10, 13 and 19 in Tables 1 and 2) were analyzed because of the limited amount of total RNA extracted from each patient. In all cases, sample margins contained no sign of tumor invasion, so the samples were considered to be tumor-free. In addition, samples of insulinoma (*n*=2), glucagonoma (*n*=1) and a pancreatic acinar cell tumor (*n*=1) were collected at surgery. From another patient with a glucagonoma, samples of normal pancreatic tissue and glucagonoma were obtained as formalin-fixed, paraffin-embedded (FFPE) sections. Islets were promptly isolated from pancreatic samples using the mince method and were collected manually using a stereomicroscope [9, 10]. In Japan, HbA1c is

measured using high-performance liquid chromatography with a set of calibrators assigned by the Japan Diabetes Society (normal range 4.3-5.8%). A correlational analysis showed that, in Japan, estimated HbA1c values are 0.4% lower than those measured by the National Glycohemoglobin Standardization Program (NGSP) [32]. For that reason, we standardized the obtained HbA1c values to NGSP units by adding 0.4% to the measured values.

2.2. Preparation and culture of mouse pancreatic islets, the MIN6 mouse insulinoma cell line and the alpha-TC mouse glucagonoma cell line

Male 14-week-old C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan) and housed in a temperature-, humidity- and light-controlled room with free access to water and standard chow (Nosan Corporation, Kanagawa, Japan). Mouse pancreatic islets were isolated as previously described [33]. All experimental procedures were approved by the Animal Research Committee, Kyoto University Graduate School of Medicine, and were performed in accordance with institutional and national guidelines for animal experimentation. MIN6 cells were kindly provided by Dr. Junichi Miyazaki [34], and alpha-TC cells were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). MIN6 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 15% FBS, while alpha-TC cells were maintained in RPMI 1640 medium supplemented with 10% FBS. Both media also contained 100 units/ml penicillin and 0.1 mg/ml streptomycin (Life Technologies

Japan, Tokyo, Japan). The cells were incubated at 37°C under an atmosphere of humidified air (95%) and CO_2 (5%).

2.3. Total RNA preparation and cDNA synthesis

Total RNAs were extracted from fresh tissues and cell lines using QIAGEN RNeasy Mini Kits [9, 10, 33], and from FFPE tissue sections using QIAGEN RNeasy FFPE Kits (QIAGEN K.K., Tokyo, Japan). The collected RNA was then treated with DNase I to remove any contaminating DNA. Additionally, total RNAs from human brain, thyroid, heart, lung, trachea, kidney, esophagus, liver, skeletal muscle, adipose tissue, spleen, bladder, prostate, placenta and cervix were obtained from Life Technologies Japan. Total RNAs from stomach, small intestine, colon, pancreas, testis, ovary and uterus were from Takara Clontech (Tokyo, Japan). Finally, total RNAs from hypothalamus were obtained from two sources, Life Technologies Japan and BioChain Institute (Hayward, CA, USA). First strand cDNA was synthesized by random hexamer-primed reverse transcription using SuperScript II reverse transcriptase (Life Technologies Japan).

2.4. Quantification of human and mouse receptor gene expression

Levels of GPR119 mRNA in the pancreas and pancreatic islets were compared with those of GPR40, the glucagon-like peptide-1 receptor (GLP1R) and the sulfonylurea receptor 1

143 (ABCC8 or SUR1) mRNA, which are reportedly expressed in human pancreatic islets and
144 involved in insulin secretion [9, 10]. Messenger RNA levels were quantified using the
145 TaqMan PCR method with an ABI PRISM 7700 Sequence Detector (Life Technologies
146 Japan), as described previously [9, 10]. To estimate the copy number of each mRNA,
147 standard curves were generated using oligo DNA fragments (Sigma Genosys Japan, Tokyo,
148 Japan) containing the PCR amplicon region. The receptor mRNA levels were normalized to
149 the level of GAPDH mRNA and expressed as the receptor/GAPDH [copy/copy] ratio [9]. The
150 sequences of the primers and probes (Life Technologies Japan) used for the quantification of
151 the mRNAs were as follows: human *GPR119* (NM_178471),
152 CCATGGCTGGAGGTTATCGA (forward), GCTCCCAATGAGAACAGACACA (reverse)
153 and 6-carboxyfluorescein
154 (FAM)-CCCCACGGACTCCCAGCGACT-6-carboxytetramethylrhodamine (TAMRA)
155 (probe); mouse *GPR119* (NM_181751), TCCAGAGAGGACCAGAGAAAGC (forward),
156 GCAGCGTCTTAGCCATCGA (reverse) and
157 FAM-TCACATCGTCACTATCAGCCATCCGG-TAMRA (probe); mouse *GPR40*
158 (NM_194057), GGCTTTCCATTGAACTTGTTAGC (forward),
159 CCCAGATGGAGAGTGTAGACCAA (reverse) and
160 FAM-TGTCCCACGCTAAACTGCGACTCACTC-TAMRA (probe); mouse *GADPH*
161 (NM_008084), TCCATGCCATCACTGCCA (forward), GCCCCACGGCCATCA (reverse)

and FAM-CAGAAGACTGTGGATGGCCCCTC-TAMRA (probe). The sequences of the primers and probes used for quantification of the human GPR40, GLP1R, ABCC8 (SUR1) and GAPDH mRNAs are described elsewhere [9, 10].

2.5. Data analysis on metabolic parameters

We evaluated beta cell function and systemic insulin resistance using the insulinogenic index (*n*=10) [35] or the homeostasis model assessment of beta cell function (HOMA-beta) (*n*=14) and insulin resistance (HOMA-IR) (*n*=14) [36], respectively. The difference between the numbers of patients whose test data were included in the HOMA indices and insulinogenic index reflects the availability of data for plasma glucose and serum insulin levels at the 30 min mark during the oral glucose tolerance test (OGTT). The area under the serum insulin concentration-time curve (insulin AUC) was calculated from the OGTT data using the trapezoidal rule. Patients 7, 12 and 17 were excluded from analysis of the correlation between pancreatic GPR119 mRNA levels and metabolic parameters, because of a diagnosis of insulinoma (patient 7) or percutaneous transhepatic biliary drainage (patients 12 and 17). None of the patients were treated with oral glucose-lowering agents or with insulin. Table 2 shows the metabolic parameters of the patients whose pancreatic tissues were examined; the patient numbers correspond to those in Table 1.

2.6. Statistical analysis

Correlations between pancreatic GPR119 mRNA levels and clinical parameters were examined using the simple regression analysis. Differences between groups were assessed using unpaired two-tailed *t*-tests or ANOVA where applicable. Values of $p < 0.05$ were considered significant (Statcel, Social Research Information, Tokyo, Japan).

3. Results

3.1 Expression of GPR119 mRNA in normal human tissues

We initially tested for GPR119 mRNA in samples of commercially available total RNA from normal human tissues. We found that the transcript was most abundant in the pancreas, followed by the gastrointestinal tract (small intestine, colon and stomach) and the testis (Fig. 1A). GPR119 mRNA was not detected in any other human tissue tested. To gain further insight into *GPR119* gene expression humans and verify the aforementioned distribution profile, we also examined tissues obtained at surgery or autopsy. Among those samples, GPR119 mRNA was most abundant in the pancreas, followed by the duodenum, stomach, jejunum, ileum and colon, but was not detected in the esophagus, liver or cerebrum (Fig. 1B).

3.2 Expression of GPR119, GPR40, GLP1R and SUR1 mRNAs in the human pancreas

Using specimens from four patients, we compared the pancreatic expression of GPR119 mRNA with that of GPR40, GLP1R and SUR1 mRNA in the same samples. We found that pancreatic levels of GPR119 mRNA were comparable to those of GPR40 mRNA and were higher than those of GLP1R and SUR1 mRNA (Fig. 1C).

3.3. Expression of GPR119 and GPR40 mRNA in isolated pancreatic islets and adjacent pancreatic tissue

We next assessed *GPR119* expression in pancreatic islets from three patients (Fig. 2A). Levels of GPR119 mRNA in freshly isolated islets were approximately 13 to 16 times higher than in the adjacent pancreatic tissue from the same patients. We also analyzed *GPR40* expression and found that levels of GPR119 and GPR40 mRNA were similar in isolated pancreatic islets (Fig. 2B).

3.4. Expression of GPR119 and GPR40 mRNA in human insulinomas and glucagonomas

We also assessed expression of GPR119 and GPR40 mRNA using total RNAs extracted from specimens of fresh insulinomas (*n*=2), a glucagonoma (n=1) and a pancreatic acinar cell tumor (*n*=1), as well as from FFPE glucagonoma tissue sections from another patient (*n*=1). In the two cases of insulinoma, tumoral GPR119 mRNA levels were comparable to those in pancreatic islets (Fig. 3A). A considerable amount of GPR119 mRNA was also detected in tissue extracts from the glucagonoma (Fig. 3A), where GPR40 mRNA was not detectable (Fig. 3C). Levels of GPR119 mRNA in tissue extracts from FFPE sections of non-tumor pancreas and glucagonoma were similar to those in the corresponding specimens collected at surgery (Fig. 3, A and B). GPR40 mRNA was not detected in extracts from the same FFPE

glucagonoma sections (Fig. 3D), which is consistent with the level in the fresh tumor specimen (Fig. 3, C and D). Neither GPR119 nor GPR40 mRNAs was detectable in the acinar cell tumor specimen (Fig. 3, A and C).

3.5. Expression of GPR119 and GPR40 mRNAs in mouse pancreatic islets, MIN6 insulinoma cells and alpha-TC glucagonoma cells

To further explore *GPR119* expression in pancreatic islet cells, we measured GPR119 mRNA levels in mouse pancreatic islets, MIN6 insulinoma cells and alpha-TC glucagonoma cells. We also assessed expression of GPR40 mRNA in the same samples, as GPR40 is known to be preferentially expressed in pancreatic beta cells in both rodents and humans [4, 9, 10, 37]. High levels of GPR119 mRNA, comparable to those of GPR40 mRNA, were detected in mouse pancreatic islets (Fig. 4, A and B). Likewise, similar levels of GPR119 and GPR40 mRNA were detected in MIN6 cells (Fig. 4, A and B). On the other hand, the level of GPR119 mRNA in alpha-TC cells was approximately 1/7 that in MIN6 cells, and no GPR40 mRNA was detected in alpha-TC cells (Fig. 4, A and B).

3.6. Correlation between pancreatic GPR119 mRNA expression and the insulinogenic index and HOMA-beta in humans

To investigate the functional implications of pancreatic GPR119 expression in humans, we

initially assessed GPR119 mRNA expression in non-tumor pancreatic tissue samples from 19 patients with various pancreatic tumors (Table 1). High levels of GPR119 mRNA, comparable to those in the four cases summarized in Fig 1, A and B (0.336±0.037 *vs.* 0.319±0.090), were detected in all of the tissue samples analyzed (Table 2). Because the inter-individual variation in the pancreatic GPR119 mRNA level (n=19) was high, to begin to explore the physiological importance of GPR119 in humans, we evaluated the relationship between pancreatic GPR119 mRNA levels and various clinical parameters. We found that GPR119 mRNA expression did not significantly differ among the head, body and tail portions of the pancreas (Table 3), nor did it correlate significantly with age (Supplemental Table S1).

When we then evaluated the correlation between pancreatic *GPR119* gene expression and several metabolic parameters, including glucose and triglyceride metabolism (Table 2), we found that pancreatic GPR119 mRNA levels did not correlate significantly with BMI, fasting plasma glucose (FPG), 2-h post-OGTT plasma glucose (2h-PG), insulin AUC or fasting serum triglyceride levels (Supplemental Table S1), nor did they correlate significantly with HbA1c levels or HOMA-IR values (Supplemental Table S1, Fig. 5, A and B). By contrast, pancreatic GPR119 mRNA levels positively and significantly correlated with the insulinogenic index (n=10, p=0.004, r=0.817) (Fig. 5C) and with HOMA-beta values (n=14, p=0.043, r=0.547) (Fig. 5D). Using the same patient data used to calculate the insulinogenic index (n=10) and HOMA-beta (n=14), we also tested for correlations between GPR119

mRNA expression and HbA1c levels and HOMA-IR values, which confirmed the absence of a significant correlation (Supplemental Table S1).

4. Discussion

Our findings demonstrate for the first time that *GPR119* is highly expressed in human pancreatic islets, where the level of *GPR119* expression is enriched more than 10-fold, as compared to adjacent areas of the pancreas in the same individuals. We also found that pancreatic levels of GPR119 mRNA are similar to those of GPR40 mRNA and are higher than those of GLP1R and SUR1 mRNA. Likewise, the level of GPR119 mRNA in isolated pancreatic islets is similar to that of GPR40 mRNA and higher than those of SUR1 and GLP1R mRNA [9, 10]. This is noteworthy, as these receptors are reported to be abundantly expressed in human pancreatic islets.

We observed that substantial amounts of GPR119 mRNA are expressed in human insulinomas (n=2) and glucagonomas (n=2), and that the tumoral levels of the transcript are comparable to those in pancreatic islets. A similar pattern of GPR119 mRNA expression was also detected with mouse pancreatic islets, MIN6 insulinoma cells and alpha-TC glucagonoma cells. Thus GPR119 appears to be highly expressed in both beta and alpha cells in human and mouse pancreatic islets. Moreover, our observation that the expression levels of GPR119 and GPR40 mRNAs in human pancreatic islets are similar and are higher than that of SUR1 mRNA is noteworthy because SUR1 is reported to be abundantly expressed in both beta and alpha cells and is involved in the regulation of islet function, including insulin and glucagon secretion [38-40]. This strong expression suggests GPR119 may be involved in

pancreatic islet function in humans. Consistent with that idea, pancreatic GPR119 mRNA levels correlated positively with two indices of beta cell function: the insulinogenic index and the HOMA-beta. Collectively, therefore, the present findings provide evidence for the possible involvement of GPR119 in islet function, perhaps affecting insulin secretion.

Using fresh tissue samples collected at surgery, we observed that, in humans, GPR119 mRNA is abundantly expressed in the small intestine, stomach and colon, but not in the esophagus. In rodents, GPR119 appears to be expressed in enteroendocrine cells, including L and K cells, and to be involved in the regulation of incretin and polypeptide YY secretion. In humans, enteroendocrine cells are distributed throughout the gastrointestinal tract, but not in the esophagus. Although details of GPR119 expression and its function in the human gastrointestinal tract will require further investigation, our findings are consistent with the idea that GPR119 is expressed in enteroendocrine cells and is involved in incretin and peptide YY secretion.

We detected no GPR119 mRNA in the human hypothalamus, brain or cerebrum, which is consistent with a recent report that GPR119 mRNA is not significantly expressed in the human brain or hypothalamus [19]. Although earlier reports using OEA (a putative GPR119 ligand) and a synthetic OEA analogue in rats suggest GPR119 may mediate signalling leading to reduced food intake and body weight, OEA appears to act mainly in peripheral tissues, rather than in the central nervous system [41]. Our finding that GPR119 mRNA is

highly expressed in the human stomach and duodenum is consistent with the notion that GPR119 is involved in regulating food intake in humans, as the bipolar vagal afferents involved in regulating feeding are known to project to the stomach and upper intestine [42, 43].

In summary, the present study demonstrates that, in humans, GPR119 mRNA is abundantly expressed in healthy pancreatic islets and the human gastrointestinal tract, and in insulinomas and glucagonomas. The results provide evidence of an islet-gastrointestinal distribution of GPR119, its expression in pancreatic beta and alpha cells, and its possible involvement in islet function. They also provide the basis for a better understanding of the potential clinical importance of GPR119.

4.1. Limitations of the present study

Our study has several limitations that should be noted.

1. To our knowledge, no specific antibody against human GPR119 is available, so we were unable to assess expression of GPR119 protein.
2. The enrolled subjects were tumor-bearing patients, though the tumors were at an early stage or benign, and were resectable. Pancreatic biopsy is rarely performed because of the risk of pancreatitis, and is not justified in those without severe illness [44]. Therefore, we analyzed human pancreatic tissues collected during surgery. Because

pancreatic tissue is very vulnerable to postmortem autolysis, specimens obtained at surgery offer substantial advantages for precise analysis of *GPR119* expression. Nonetheless, possible weight loss and the paracrine effects of pancreatic cancer cells on beta cells could have influenced the correlation study.

3. Patients enrolled in the present study were not severely diabetic (HbA1c was less than 7.2%), nor were they overweight or obese (BMIs were less than 25). Thus clarification of the pathophysiological role of GPR119 in human diabetes and obesity must await further investigation in patients with a wider range of glucose tolerances and BMIs.

4. Plasma glucagon levels were not determined in the preoperative evaluation, and were not included in the present study. Beta cell mass is known to be much greater than alpha cell mass in pancreatic islets, and correlations between GPR119 mRNA levels and indices for beta cell function seem plausible, but may underestimate the involvement of GPR119 in the glucagon secretion. Further studies will be necessary to clarify the role of GPR119 in glucagon secretion.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author contributions:

SO: data collection and analysis, data interpretation, manuscript writing. KH: data interpretation, manuscript writing. TT: data analysis, data interpretation, manuscript writing. JF, TK and KE: data interpretation, manuscript writing. YK, RD, KT, YS and SU: data

355 collection, data interpretation. KN: data interpretation, manuscript writing.

Figure Legends

Fig. 1 - Expression of GPR119 mRNA in human tissues. All receptor mRNA levels were normalized to the level of GAPDH mRNA in the same tissue. A, GPR119 mRNA levels in commercially obtained samples of human total RNA from the indicated tissues. B, GPR119 mRNA expression in the indicated human tissues collected at autopsy (cerebrum) or at surgery (all tissues except cerebrum). C, Expression of GPR119, GPR40, GLP1R and SUR1 mRNA in normal human pancreatic tissue collected at surgery (*n*=4). The specimens used were the same as in panel B. Receptor mRNA levels in panels B and C are expressed as means ± SEM. *Black bar*, GPR119; *white bar*, GPR40; *hatched bar*, GLP1R; *double-hatched bar*, ABCC8 (SUR1).

Fig. 2 - Expression of GPR119 mRNA in human pancreatic islets and adjacent pancreatic tissue. All receptor mRNA levels were normalized to the level of GAPDH mRNA in the same tissue. A, Comparison of GPR119 mRNA expression in pancreatic islets and adjacent pancreatic tissue from three patients. *White bars*, pancreas; *black bars*, pancreatic islets. B, Comparison of GPR119 and GPR40 mRNA expression in pancreatic islets and adjacent pancreatic tissue. The tissue samples used were the same as in panel A (*n*=3). Levels of GPR119 and GPR40 mRNA are expressed as means ± SEM. *White bars*, pancreas; *black bars*, pancreatic islets.

375

376 Fig. 3 - Expression of GPR119 and GPR40 mRNA in human pancreatic islets, insulinomas
377 and glucagonomas. A and C, Expression of GPR119 (A) and GPR40 (C) mRNA in non-tumor
378 pancreas (Pancreas), pancreatic islets (Islets), insulinomas (INS), a glucagonoma (GLU) and
379 a pancreatic acinar cell tumor (ACI). B and D, Expression of GPR119 (B) and GPR40 (D)
380 mRNA in extracts from non-tumor pancreatic and glucagonoma tissue sections (*n*=1 each).
381 All receptor mRNA levels were normalized to the level of GAPDH mRNA in the same tissue.
382 GPR119 and GPR40 mRNA levels in pancreas and pancreatic islets are expressed as means ±
383 SEM. *White bars*, pancreas; *black bars*, pancreatic islets; *hatched bars*, insulinomas;
384 *double-hatched bars*, glucagonomas.

385

386 Fig. 4 - Expression of GPR119 and GPR40 mRNAs in mouse pancreatic islets, insulinoma
387 and glucagonoma. Expression of GPR119 (A) and GPR40 (B) mRNAs pancreatic islets,
388 MIN6 insulinoma cells and alpha-TC glucagonoma cells. All receptor mRNA levels were
389 normalized to the level of GAPDH mRNA in the same tissue. *Black bars*, pancreatic islets;
390 *hatched bars*, MIN6 cells; *double hatched bars*, alpha-TC cells.

391

392 Fig. 5 - Correlations between human pancreatic GPR119 mRNA levels and parameters of
393 glucose metabolism, including HbA1c levels (*n*=16) (A), HOMA-IR values (*n*=14) (B), the

394 insulinogenic index (n=10) (C) and HOMA-beta values (n=14) (D). All GPR119 mRNA
395 levels were normalized to the level of GAPDH mRNA in the same tissue. Simple regression
396 analysis was used to determine p and r values. The solid lines are regression lines.

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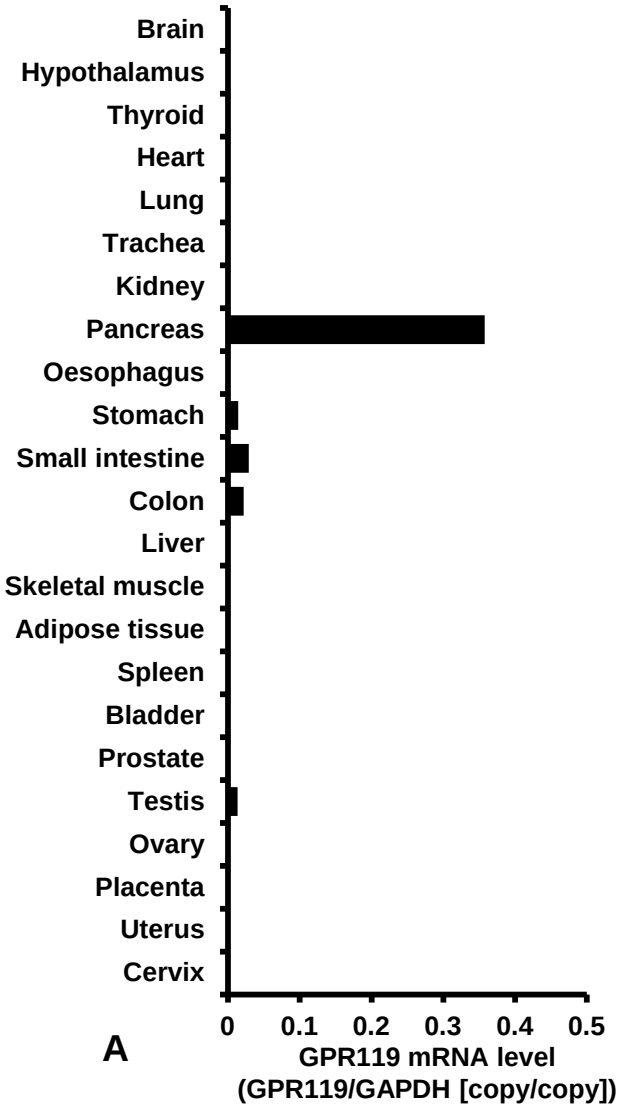
Fig.1


Brain
Hypothalamus
Thyroid
Heart
Lung
Trachea
Kidney
Pancreas
Oesophagus
Stomach
Small intestine
Colon
Liver
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Adipose tissue
Spleen
Bladder
Prostate
Testis
Ovary
Placenta
Uterus
Cervix
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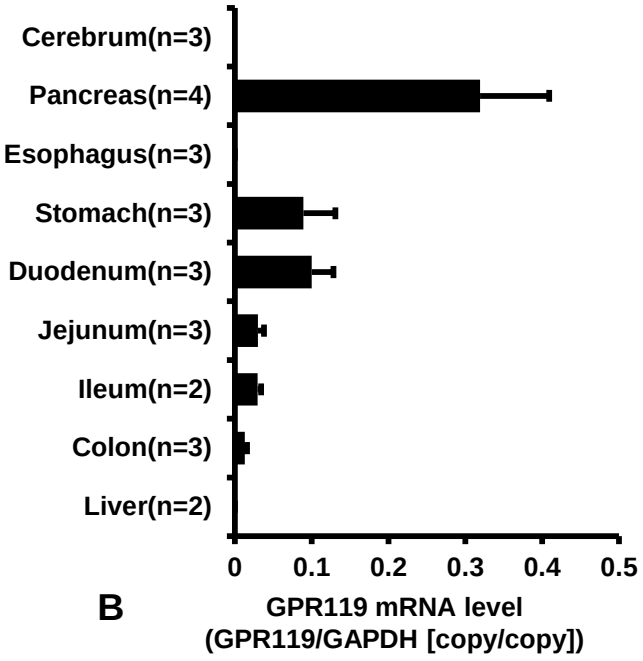
0.1
0.2
0.3
0.4
0.5
A
GPR119 mRNA level
(GPR119/GAPDH [copy/copy])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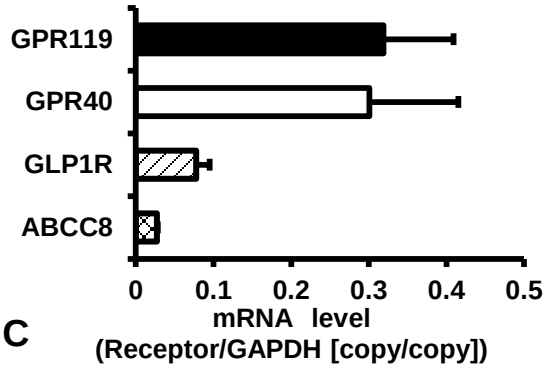
Pancreas(n=4)
Esophagus(n=3)
Stomach(n=3)
Duodenum(n=3)
Jejunum(n=3)
Ileum(n=2)
Colon(n=3)
Liver(n=2)
0
0.1
0.2
0.3
0.4
0.5
B
GPR119 mRNA level
(GPR119/GAPDH [copy/copy])


GPR119
GPR40
GLP1R
ABCC8
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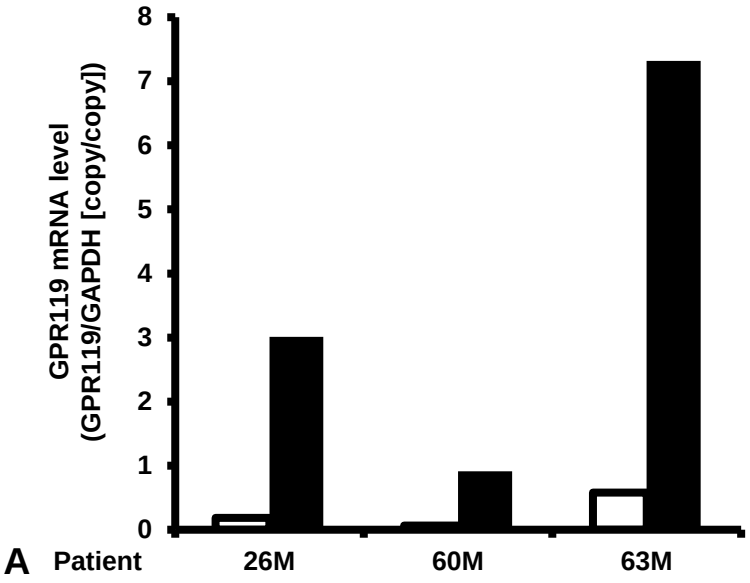
0.1
0.2
0.3
0.4
0.5
C
mRNA level
(Receptor/GAPDH [copy/copy])

Fig.2


GPR119 mRNA level
(GPR119/GAPDH [copy/copy])
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7
6
5
4
3
2
1
0
A
Patient
26M
60M
63M

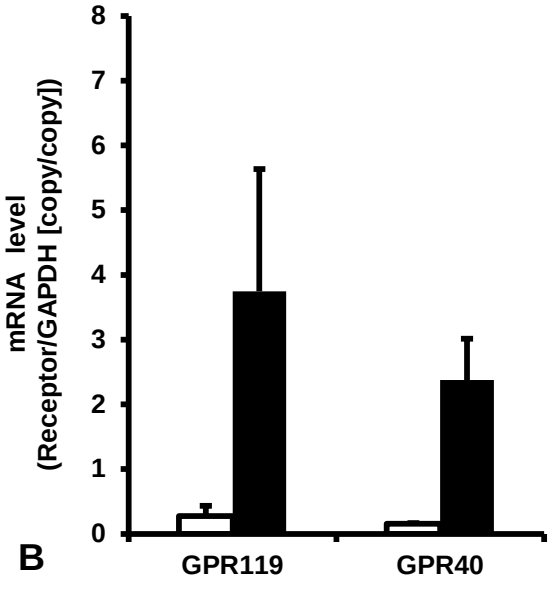
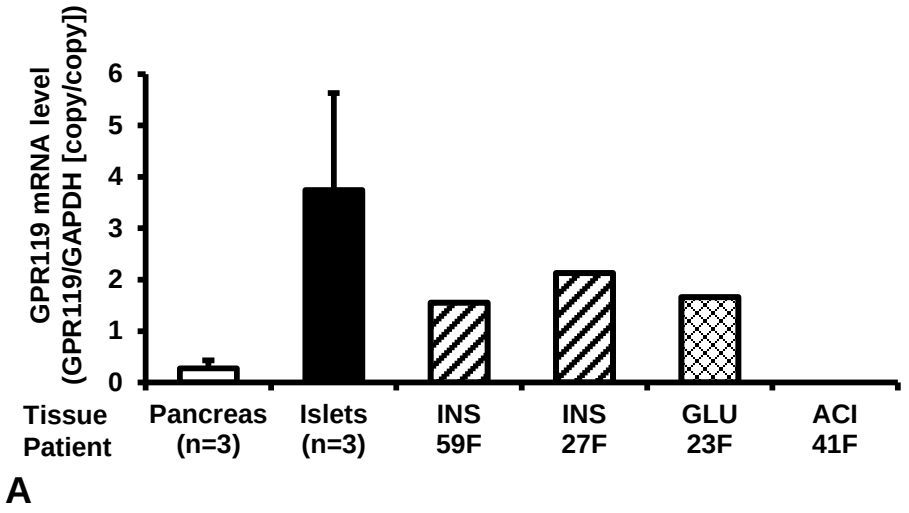

mRNA level
(Receptor/GAPDH [copy/copy])
8
7
6
5
4
3
2
1
0
B
GPR119
GPR40

Fig.3


GPR119 mRNA level
(GPR119/GAPDH [copy/copy])
6
5
4
3
2
1
0
Tissue
Patient
Pancreas
(n=3)
Islets
(n=3)
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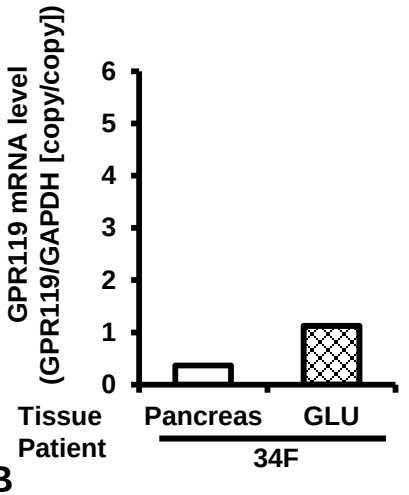
59F
INS
27F
GLU
23F
ACI
41F

A


GPR119 mRNA level
(GPR119/GAPDH [copy/copy])
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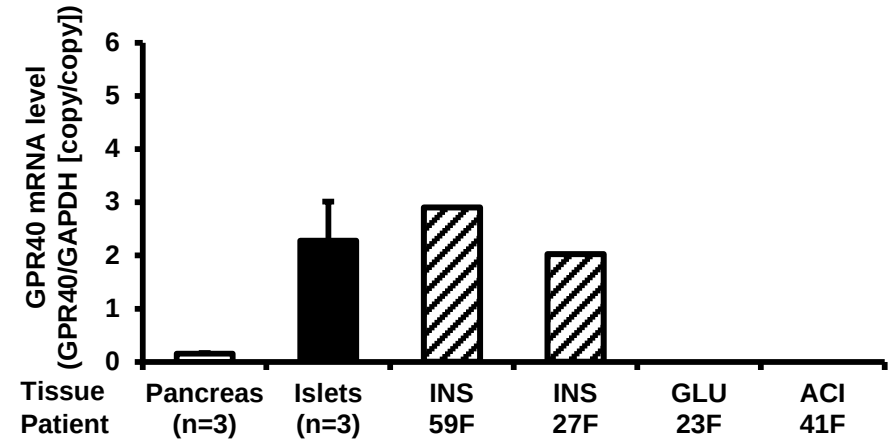
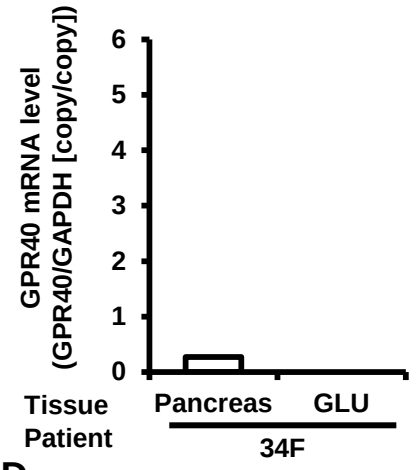
5
4
3
2
1
0
Tissue
Patient
Pancreas
GLU
34F

B

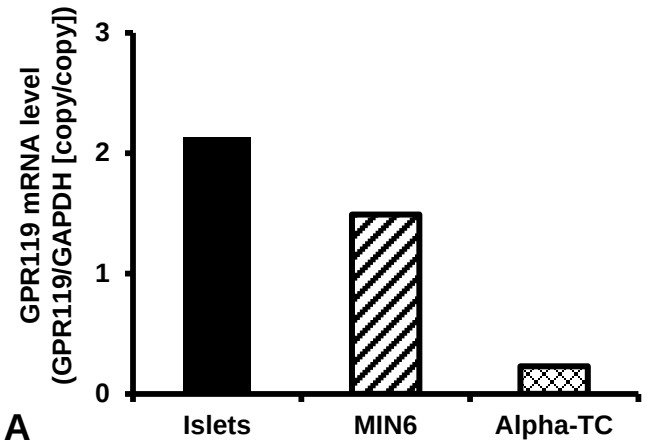

GPR40 mRNA level
(GPR40/GAPDH [copy/copy])
6
5
4
3
2
1
0
Tissue
Patient
Pancreas
(n=3)
Islets
(n=3)
INS
59F
INS
27F
GLU
23F
ACI
41F

C


GPR40 mRNA level
(GPR40/GAPDH [copy/copy])
6
5
4
3
2
1
0
Tissue
Patient
Pancreas
GLU
34F

D

Fig.4


GPR119 mRNA level
(GPR119/GAPDH [copy/copy])
3
2
1
0
Islets
MIN6
Alpha-TC
A

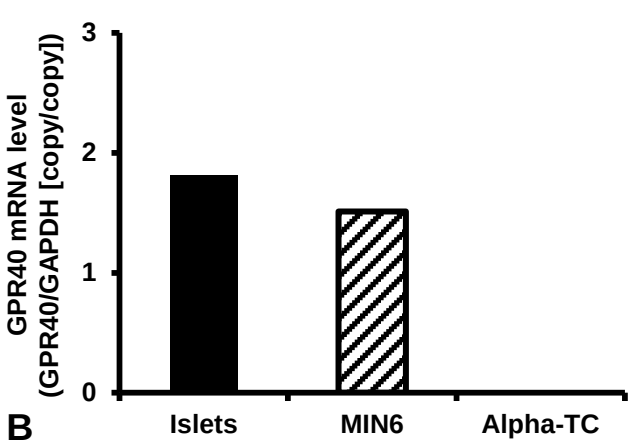

GPR40 mRNA level
(GPR40/GAPDH [copy/copy])
3
2
1
0
Islets
MIN6
Alpha-TC
B

Fig.5

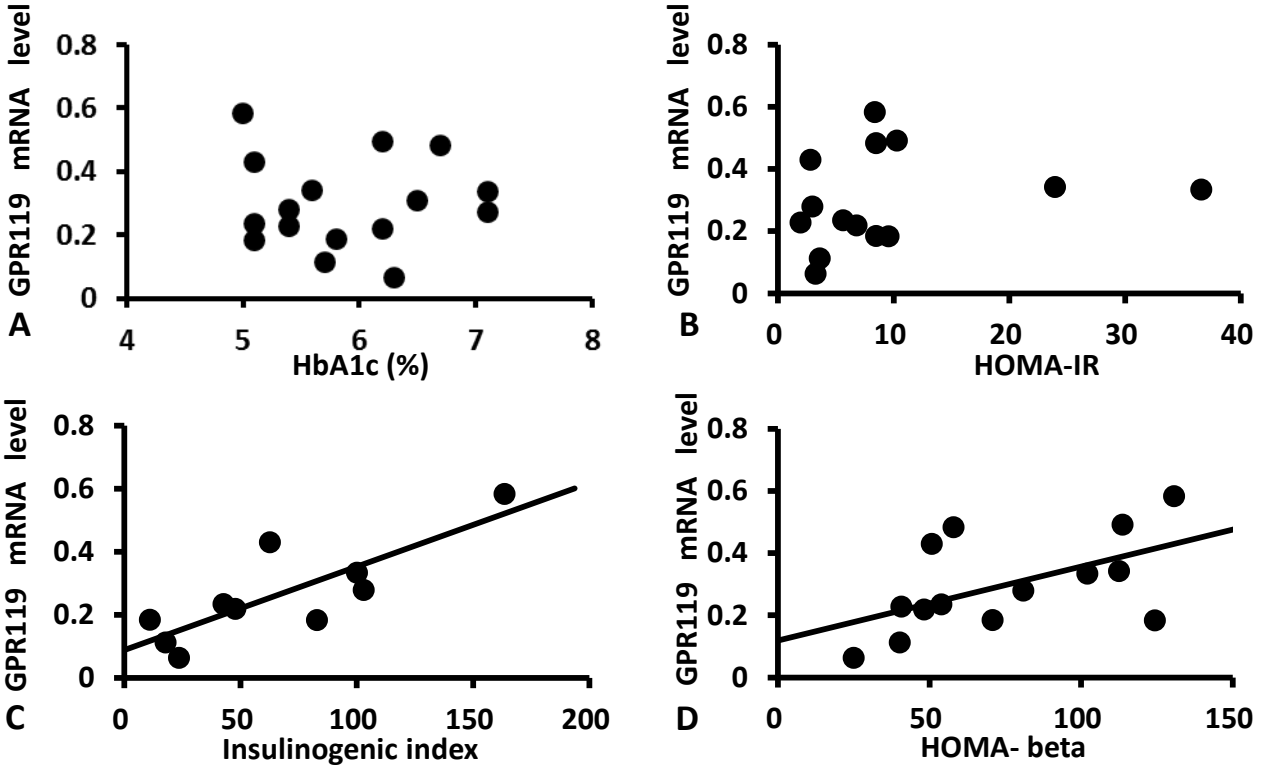

GPR119 mRNA level
0.8
0.6
0.4
0.2
0
A
4
5
6
7
8
HbA1c (%)
B
0
10
20
30
40
HOMA-IR
C
0
50
100
150
200
Insulinogenic index
D
0
50
100
150
HOMA- beta

Table 1. Clinical profiles of the patients who underwent pancreatectomy and tissues analyzed

Patient	Age(years)	Sex(M/F)	Disease	Tissue analyzed
1	26	M	Pancreatic cancer	Pancreas (tail)
2	47	F	Pancreatic cancer	Pancreas (head)
3	53	F	Pancreatic cancer	Pancreas (head)
4	54	M	Pancreatic cancer	Pancreas (head)
5	55	F	Pancreatic cancer	Pancreas (body)
6	57	M	Islet cell tumor (nonfunctional)	Pancreas (tail)
7	59	F	Insulinoma	Pancreas (head), insulinoma
8	60	M	Pancreatic cancer	Pancreas (body)
9	60	M	Pancreatic cancer	Pancreas (head)
10	61	F	Papilla cancer	Pancreas (head)
11	63	F	Islet cell tumor (nonfunctional)	Pancreas (body)
12	63	M	Pancreatic cancer	Pancreas (head)
13	64	M	Pancreatic cancer	Pancreas (body)
14	69	M	Pancreatic cancer	Pancreas (head)
15	71	F	Pancreatic cancer	Pancreas (body)
16	72	F	Pancreatic cancer	Pancreas (body)
17	72	F	Pancreatic cancer	Pancreas (head)
18	75	M	Pancreatic cancer	Pancreas (head)
19	76	M	Duodenal cancer	Pancreas (head)
20	27	F	Insulinoma	Insulinoma
21	23	F	Glucagonoma	Glucagonoma
22	41	F	Acinar cell tumor	Acinar cell tumor
23	34	F	Glucagonoma	Glucagonoma

Patients were premedicated with 0.01 mg/kg atropine sulfate i.m. and 0.2 mg/kg diazepam orally before surgery. Tissues were sampled under general

anesthesia with 35% O_2, 65% N_2O and 0.5-1.5% sevoflurane. Neuromuscular blockade was provided by vecuronium bromide at an initial dose 0.1 mg/kg and supplemented as required.

Table 2. The metabolic parameters and the levels of GPR119 mRNA in the pancreas of 19 patients

Patient	BMI (kg/m^2)	FPG (mmol/l)	2h-PG (mmol/l)	Insulin AUC ($\times 10^3$ pmol/l)	HbA1c (%)	HOMA-IR	Insulinogenic index	HOMA-beta	Triglycerides (mmol/l)	GPR119 mRNA level
1	24.2	4.7	6.7	32	5.1	9.6	83.0	124.4	1.23	0.183
2	19.7	7.2	12.6	53	7.1	36.6	100.1	102.1	2.26	0.334
3	17.7	4.4	6.8	24	5.1	2.8	62.7	50.8	1.54	0.430
4	22.3	4.9	9.1	18	5.7	3.7	17.8	40.3	0.89	0.112
5	24.6	5.1	8.3	25	5.1	5.7	42.8	54.0	1.20	0.235
6	25.7	6.1	*ND*	*ND*	5.6	24.0	*ND*	112.60	1.48	0.342
7*	22.1	2.0	4.9	84	4.7	2.5	*ND*	-61.3	0.86	0.419
8	18.0	5.3	10.8	12	6.3	3.3	23.6	25.1	1.76	0.063
9	19.6	4.3	11.4	*ND*	5.4	2.0	*ND*	40.8	2.01	0.228
10	20.0	4.6	6.9	24	5.0	8.4	163.7	130.7	1.40	0.583
11	22.8	5.5	13.6	*ND*	6.7	8.5	*ND*	58.0	2.28	0.483
12†	24.2	4.8	*ND*	*ND*	6.5	*ND*	*ND*	*ND*	1.01	0.582
13	23.3	5.2	10.7	18	5.8	8.5	11.1	70.8	1.29	0.185
14	24.3	*ND*	*ND*	*ND*	7.1	*ND*	*ND*	*ND*	0.95	0.272
15	23.5	4.9	8.9	*ND*	6.2	10.3	*ND*	113.8	1.99	0.492
16	18.4	6.1	*ND*	*ND*	6.5	*ND*	*ND*	*ND*	2.03	0.306
17†	16.8	5.1	*ND*	*ND*	5.8	*ND*	*ND*	*ND*	1.02	0.631
18	22.6	5.4	8.3	51	6.2	6.8	47.8	48.3	1.60	0.219
19	20.3	4.2	6.8	48	5.4	3.0	103.1	81.0	0.49	0.279

The patient numbers correspond to those in Table 1. *Patient 7 was diagnosed as having an insulinoma. †Patients 12 and 17 were treated with percutaneous transhepatic biliary drainage (PTBD). Because of the unavailability of blood samples, some of the metabolic profiles were not determined (shown as *ND*). *FPG*, fasting plasma glucose level; *2h-PG*, 2-h post-OGTT plasma glucose level; *ND*, not determined

Table 3. GPR119 mRNA levels in various regions of the pancreas in humans

Pancreatic region(s)	GPR119 mRNA level	*n*	*p**
Head	0.372 ± 0.052	11	-
Body	0.294 ± 0.069	6	0.388
Tail	0.262 ± 0.079	2	0.367
Body and tail	0.286 ± 0.053	8	0.264

GPR119 mRNA levels are expressed as means ± SEM. Comparisons were made using unpaired two-tailed *t*-tests. **p* values are *vs* the head.

Supplemental Table S1: Correlation between pancreatic GPR119 mRNA levels and various clinical parameters

	n	*r*	*p*
Age	19	0.290	0.256
BMI	19	0.017	0.597
FPG	15	0.0001	0.967
2h-PG	13	0.005	0.825
Insulin-AUC	10	0.042	0.570
Triglyceride	19	0.0004	0.939
HbA1c	10	0.109	0.350
	14	0.000048	0.981
HOMA-IR	10	0.039	0.583
	14	0.047	0.454

The correlations between pancreatic GPR119 mRNA levels and various parameters were examined using simple regression analysis. FPG, fasting plasma glucose level; 2h-PG, 2-h post-OGTT plasma glucose level.