Table 3 Metabolic profiles of patients studied

Patient	BMI (kg/m^2)	FPG (mmol/l)	2h-PG (mmol/l)	Insulin AUC ($\times 10^3$ pmol/l)	HOMA-IR	Insulinogenic index	HbA_{1c} (%)	Triglycerides (mmol/l)
1	17.7	4.4	6.8	41.8	2.8	62.7	4.7	1.54
2	19.7	7.2	12.6	102.2	36.6	100.1	6.7	2.26
3	23.5	4.9	8.9	280.8	10.3	ND	5.8	1.99
4[a]	22.1	2	4.9	ND	2.5	ND	4.3	0.86
5	18.4	6.1	ND	ND	ND	ND	6.1	2.03
6	22.6	5.4	8.3	103.0	6.8	47.8	5.8	1.60
7	21.6	5.7	8.5	82.8	11.6	51.0	5.5	1.60
8	22.8	5.5	13.6	78.5	8.5	ND	6.3	2.28
9	18	5.3	10.8	22.2	3.3	23.6	5.9	1.76
10	23	4.9	ND	ND	ND	ND	5.1	1.33
11	22.3	4.9	9.1	36.0	3.7	17.8	5.3	0.89
12	24.6	5.1	8.3	44.9	5.7	42.8	4.7	1.20

[a]Patient 4 was diagnosed as having insulinoma
Because of the unavailability of blood samples, some of the metabolic profiles were not determined (shown as ND)
2h-PG Plasma glucose at 2 h under the OGTT, *FPG* fasting plasma glucose, *ND* not determined

Discussion

GPR40 is abundantly expressed in murine pancreatic beta cells [3, 7] where it mediates the fatty acid-induced augmentation of GSIS in vitro [6]. Long-chain fatty acids act as ligands for human GPR40 in vitro [3, 7, 23]. Additionally, two studies suggest the possible involvement of GPR40 in the proliferation and cell function of breast cancer [27, 28]. Two laboratories reported the possible relationship between variation of the *GPR40* single-nucleotide polymorphisms and insulin secretion in humans [22, 23], where the results were inconsistent. The physiological role of GPR40 in humans remains obscure.

We here demonstrate for the first time that a large amount of *GPR40* mRNA is expressed in pancreatic islets in humans using expeditiously isolated islets from pancreatic tissue. TaqMan analysis revealed that levels of *GPR40* mRNA expression in pancreatic islets were 20-fold higher than those in the pancreas in the same individuals. Notably, the mRNA level of the *GPR40* gene in isolated islets was comparable to those of genes encoding GLP1R, ABCC8 (SUR1) and SSTRs, all of which are abundantly expressed in human pancreatic islets [13–16].

The present study demonstrates that a large amount of *GPR40* mRNA is expressed solely in three cases of insulinoma among islet cell tumours. The finding suggests that the *GPR40* mRNA detected in insulinoma is attributed to the type of islet cell tumour rather than the inclusion of endothelial, neuronal or other types of cells. Collectively, these data prompt us to speculate that GPR40 is expressed mainly in beta cells in the human pancreatic islet.

It is noteworthy that the mRNA levels of *GPR40* and *ABCC8* (*SUR1*) were comparable in human pancreatic islets, because *ABCC8* (*SUR1*) is abundantly expressed in

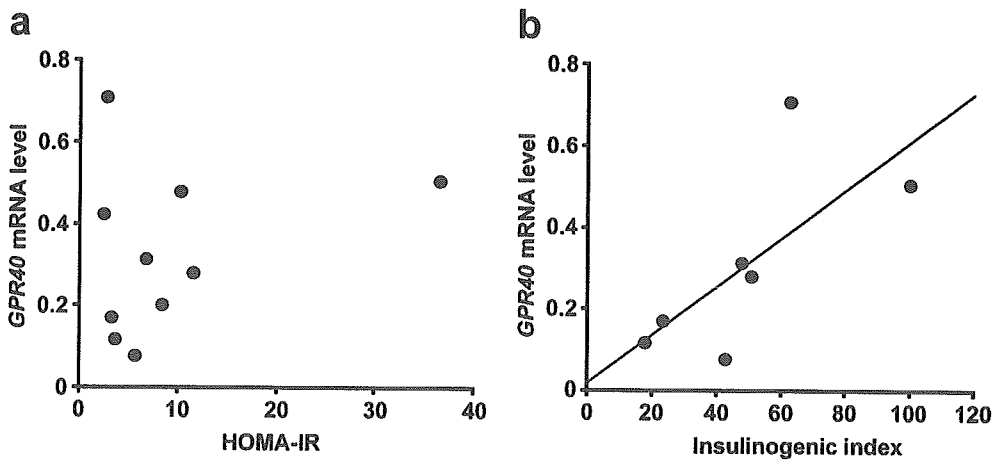

Fig. 3 Positive correlation between *GPR40* mRNA level in the pancreas and insulinogenic index. **a** Relationship between *GPR40* mRNA level in the pancreas and the HOMA-IR (n=10). *GPR40* mRNA level and the HOMA-IR did not correlate significantly (r=0.11, p=0.73). **b** Relationship between *GPR40* mRNA level in the pancreas and the insulinogenic index (n=7). Correlation was marginally but significantly positive between *GPR40* mRNA level and the insulinogenic index (r=0.82, p=0.044). Spearman's rank correlation test was used to determine p and r values. The solid line is the regression line

human pancreatic beta cells and the protein functions as a target of sulfonylurea agents [29]. These findings suggest that GPR40 has also some functional property in terms of insulin secretion in humans. In the present study, the *GPR40* mRNA level in the pancreas significantly correlated with the insulinogenic index rather than the HOMA-IR, supporting the notion that GPR40 is involved in the regulation of insulin secretion in humans.

A recent study of *GPR40* knockout mice and beta-cell-specific *GPR40* transgenic mice provided evidence that GPR40 is involved in the pathophysiology of glucose intolerance and beta cell lipotoxicity [11]. In this context, it is important to note that patients enrolled in the present study were neither obese nor severely diabetic. Thus, clarification of the pathophysiological role of GPR40 in human diabetes must await further investigation in patients with a wider range of body weight, glucose intolerance or dyslipidaemia.

As pancreatic tissues are very vulnerable to postmortem autolysis, specimens obtained at operation have a great advantage for the precise analysis of the *GPR40* mRNA level. Pancreatic biopsy is rarely conducted because of the risk of pancreatitis and is not justified in those without severe illness [30]. Thus, we analysed human pancreatic tissues collected during surgery. To our knowledge, specific antibody against human GPR40 has not been available, hence the lack of analyses of GPR40 protein expression in the present study.

In summary, the present study demonstrates that *GPR40* mRNA is abundantly expressed in human pancreatic islets and insulinoma. The results provide evidence for GPR40 expression in pancreatic beta cells and its involvement in insulin secretion in humans.

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References

1. Nunez EA (1997) Biological complexity is under the 'strange attraction' of non-esterified fatty acids. Prostaglandins Leukot Essent Fat Acids 57:107–110
2. Corkey BE, Deeney JT, Yaney GC, Tornheim K, Prentki M (2000) The role of long-chain fatty acyl-CoA esters in beta-cell signal transduction. J Nutr 130:299S–304S
3. Briscoe CP, Tadayyon M, Andrews JL et al (2003) The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. J Biol Chem 278:11303–11311
4. McGarry JD, Dobbins RL (1999) Fatty acids, lipotoxicity and insulin secretion. Diabetologia 42:128–138
5. Unger RH (2003) Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. Endocrinology 144:5159–5165
6. Itoh Y, Kawamata Y, Harada M et al (2003) Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. Nature 422:173–176
7. Kotarsky K, Nilsson NE, Flodgren E, Owman C, Olde B (2003) A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. Biochem Biophys Res Commun 301:406–410
8. Brown AJ, Goldsworthy SM, Barnes AA et al (2003) The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem 278:11312–11319
9. Nilsson NE, Kotarsky K, Owman C, Olde B (2003) Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. Biochem Biophys Res Commun 303:1047–1052
10. Hirasawa A, Tsumaya K, Awaji T et al (2005) Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med 11:90–94
11. Steneberg P, Rubins N, Bartoov-Shifman R, Walker MD, Edlund H (2005) The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. Cell Metabolism 1:245–258
12. Cameron, JL, Mehigan DG, Harrington DP, Zuidema GD (1980) Metabolic studies following intrahepatic autotransplantation of pancreatic islet grafts. Surgery 87:397–400
13. Giannaccini G, Lupi R, Trincavelli ML et al (1998) Characterization of sulfonylurea receptors in isolated human pancreatic islets. J Cell Biochem 71:182–188
14. Dillon JS, Tanizawa Y, Wheeler MB et al (1993) Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. Endocrinology 133:1907–1910
15. Thorens B, Porret A, Buhler L, Deng SP, Morel P, Widmann C (1993) Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9–39) an antagonist of the receptor. Diabetes 42:1678–1682
16. Kumar U, Sasi R, Suresh S et al (1999) Subtype-selective expression of the five somatostatin receptors (hSSTR1-5) in human pancreatic islet cells: a quantitative double-label immunohistochemical analysis. Diabetes 48:77–85
17. Iwakura H, Hosoda K, Son C et al (2005) Analysis of rat insulin II promoter-ghrelin transgenic mice and rat glucagon promoter-ghrelin transgenic mice. J Biol Chem 280: 15247–15256
18. Iwakura H, Hosoda K, Doi R et al (2002) Ghrelin expression in islet cell tumors: augmented expression of ghrelin in a case of glucagonoma with multiple endocrine neoplasm type I. J Clin Endocrinol Metab 87:4885–4888
19. Li Y, Kishimoto I, Saito Y et al (2004) Androgen contributes to gender-related cardiac hypertrophy and fibrosis in mice lacking the gene encoding guanylyl cyclase-A. Endocrinology 145:951–958
20. Saito S, Iida A, Sekine A et al (2002) Identification of 779 genetic variations in eight genes encoding members of the ATP-binding cassette, subfamily C (ABCC/MRP/CFTR). J Hum Genet 47:147–171
21. Haga H, Yamada R, Ohnishi Y, Nakamura Y, Tanaka T (2002) Gene-based SNP discovery as part of the Japanese Millennium Genome Project: identification of 190,562 genetic variations in the human genome. Single-nucleotide polymorphism. J Hum Genet 47:605–610
22. Ogawa T, Hirose H, Miyashita K, Saito I, Saruta T (2005) GPR40 gene Arg211His polymorphism may contribute to the variation of insulin secretory capacity in Japanese men. Metabolism 54:296–299

23. Hamid YH, Vissing H, Holst B et al (2005) Studies of relationships between variation of the human G protein-coupled receptor 40 gene and type 2 diabetes and insulin release. Diabet Med 22:74–80
24. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA (2005) Beta-cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. J Clin Endocrinol Metab 90:493–500
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
26. Takahashi-Yasuno A, Masuzaki H, Miyawaki T et al (2004) Association of Ob-R gene polymorphism and insulin resistance in Japanese men. Metabolism 53:650–654
27. Yonezawa T, Katoh K, Obara Y (2004) Existence of GPR40 functioning in a human breast cancer cell line, MCF-7. Biochem Biophys Res Commun 314:805–809
28. Hardy S, St-Onge GG, Joly E, Langelier Y, Prentki M (2005) Oleate promotes the proliferation of breast cancer cells via the G protein-coupled receptor GPR40. J Biol Chem 280: 13285–13291
29. Gribble FM, Reimann F (2003) Sulphonylurea action revisited: the post-cloning era. Diabetologia 46:875–891
30. Imagawa A, Hanafusa T, Uchigata Y et al (2005) Different contribution of class II HLA in fulminant and typical autoimmune type 1 diabetes mellitus. Diabetologia 48:294–300