



## Dominant-negative inhibition of glucose-dependent insulinotropic polypeptide impairs function of $\beta$ cells in transgenic pigs

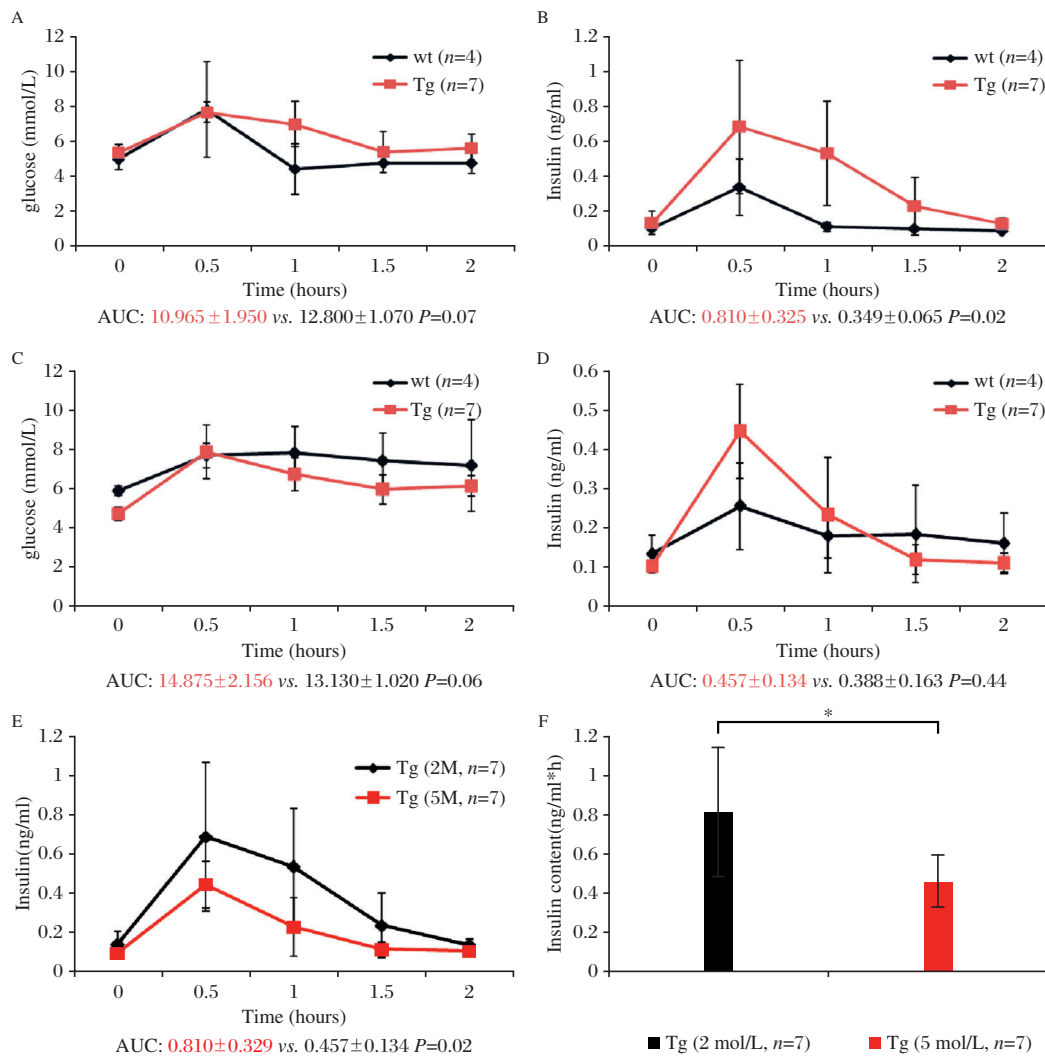
Dear Editor:

Glucose-dependent insulinotropic polypeptide (GIP) and proglucagon product glucagon-like peptide-1 (GLP-1) and their corresponding receptors promote secretion of glucose-dependent insulin<sup>[1]</sup> and may be responsible for up to 70% of postprandial insulin secretions<sup>[2]</sup>. A diminished incretin effect is typical for type 2 diabetes mellitus (T2DM) patients and their normoglycemic first-degree relatives due to reduced insulinotropic action of GIP<sup>[3]</sup>. Desensitization of the GIP/GIPR axis<sup>[4]</sup>, down-regulation of GIP receptor (GIPR) on  $\beta$  cells<sup>[5]</sup>, and variation in the *GIPR* gene<sup>[6]</sup> all contribute to reduced insulinotropic activity of GIP.

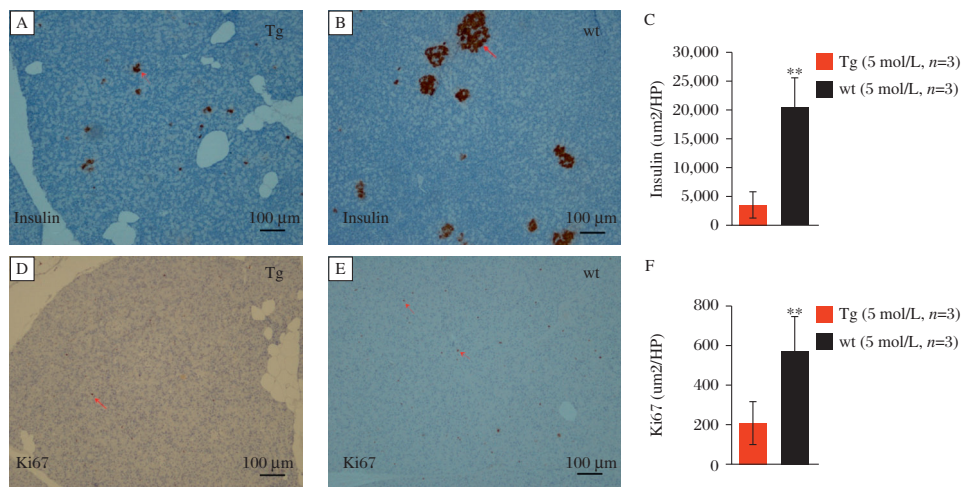
*GIPR<sup>dn</sup>* encodes human GIPR that is modified in the region of the third intracellular loop by a single mutation (Ala340→Glu340) and deletion of eight amino acids (residues 319-326) essential for signal transduction<sup>[8]</sup>. In this study, we produced porcine transgenic fibroblasts that overexpressed a *GIPR<sup>dn</sup>* under the control of rat Ins2 promoter in the pancreas. The study protocol was approved by the local institutional review board and animal study was done in accordance with established national and institutional guidelines. Oral glucose tolerance tests (OGTTs) in 2-month-old *GIPR<sup>dn</sup>* pigs and controls revealed no differences in area under the glucose response curve ( $AUC_{\text{glucose}}$ ) between the two groups ( $P=0.07$ , **Fig. 1A**). However, the postprandial blood glucose declined slowly and the area under the insulin response curve ( $AUC_{\text{insulin}}$ ) in *GIPR<sup>dn</sup>* pigs increased 2.3-fold versus the controls, implying impaired glucose tolerance ( $P=0.02$ ; **Fig. 1B**). OGTTs in 5-month-old *GIPR<sup>dn</sup>* pigs and controls showed no significant differences in glucose levels or insulin content between the groups (**Fig. 1C, 1D**). Additionally, we observed no significant differences in the insulin response profiles between 2-month-old and 5-month-old controls. However,  $AUC_{\text{insulin}}$  was reduced by 44% when the transgenic pigs grew from 2 months to 5 months of ages (**Fig. 1E, 1F**). To clarify the effect of *GIPR<sup>dn</sup>* expression on the proliferation of pancreatic cells,

we measured insulin and Ki67 expression by immunohistochemistry in 5-month-old *GIPR<sup>dn</sup>* pigs and age-matched controls. Compared with the controls, the number and volume of islets in transgenic pigs decreased. Insulin expression in the islets was reduced by 83% ( $P<0.01$ ) in 5-month-old *GIPR<sup>dn</sup>* pigs (**Fig. 2A–2C**). In addition, Ki67 expression in *GIPR<sup>dn</sup>* pigs decreased to 36% of controls, suggesting that *GIPR<sup>dn</sup>* expression impaired islet development and reduced insulin secretion (**Fig. 2D–2F**).

Transgenic mice overexpressing a dominant-negative GIPR were found to develop diabetes accompanied by a marked fasting hypoinsulinemia and severe reduction of  $\beta$ -cell mass by 10 days of age<sup>[7]</sup>. Similarly, transgenic pigs overexpressing a dominant-negative GIPR exhibited a pre-diabetic phenotype of hyperglycemia and hyperinsulinemia in OGTT<sup>[8]</sup>. However, no transgenic pigs had developed fasting hyperglycemia. In this study, 2-month-old *GIPR<sup>dn</sup>* pigs exhibited a slow decline in postprandial blood glucose and an increase in insulin secretion following an oral glucose load. This increase was approximately 2.3 times higher than in the control, thus imitating insulin secretion in the prodromal stage of T2DM. As the pigs grew up, pancreas development was significantly impaired due to the compromised GIP/GIPR axis, which resulted in reduced insulin secretion and islet dysfunction. Studies indicated that GIP and GLP-1 induced the transcription of beta cell proliferation, differentiation, survival and transcription of some genes<sup>[9]</sup>. GIP was believed to be the mitogen of beta cells, resulting in insulin secretion and inhibiting the apoptosis of beta cells. In this study, there were no significant differences in insulin content between the 5-month-old groups. However, the oral glucose load of 5-month-old transgenic pigs resulted in a 44% decrease in insulin secretion compared with the 2-month-old transgenic pigs. This is consistent with previous studies and implies progression of T2DM. Defective pancreatic proliferation led to fewer and



**Fig. 1** Oral glucose tolerance test in GIPR<sup>dn</sup> transgenic (Tg) and wild type (wt) pigs. (A) Serum glucose, and (B) insulin levels, in 2-month-old pigs (AUC glucose  $P=0.07$ , AUC insulin  $P=0.02$ ). (C) Serum glucose, and (D) insulin levels in 5-month-old pigs (AUC glucose  $P=0.06$ , AUC insulin  $P=0.44$ ). (E) (F) Insulin levels in 2-month-old and 5-month-old GIPR<sup>dn</sup> transgenic pigs ( $P=0.02$ ). \*  $P < 0.05$ .



**Fig. 2** Insulin and Ki67 expression in the pancreas. A: transgenic pigs (Tg); B: wild type (wt) pigs. Insulin is expressed in islet. C: transgenic pigs (Tg); B: wild type (wt) pigs. Ki67 expression is decreased in GIPR<sup>dn</sup> transgenic pigs. ( $n=3$  per group, \*\* $P < 0.01$ ).

smaller islets, which were excessively activated to produce more insulin by compensatory mechanism. Overload pressure from the compensation mechanism and impaired proliferation for inactivated GIP/GIPR axis further deteriorated islet function. Similar to the previous study<sup>[9]</sup>, the transgenic pigs did not develop diabetes until 2 years of age. Diabetes is caused by many factors; a single gene change would not lead to the occurrence of the disease.

In summary, GIPR<sup>dn</sup> pigs exhibit impaired glucose metabolism and beta cell function and simulated the phenotype of the prodromal stage in diabetes and progression of the disease.

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Yours Sincerely,

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