Novel murine model of congenital diabetes: The insulin hyposecretion mouse

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ABSTRACT
Aims/Introduction: Diabetic animal models have made an enormous contribution to our understanding of the etiology of diabetes and the development of new medications. The aim of the present study was to develop and characterize a novel, non-obese murine strain with spontaneous diabetes – the insulin hyposecretion (ihs) mouse.

Materials and Methods: During the development of the ICGN.B6-Tns2WT strain as the control for the ICGN-Tns2nph congenital nephrotic strain, diabetic mice were discovered and named ihs mice. Intraperitoneal insulin tolerance test, oral glucose tolerance test and an insulin secretion experiment by the pancreas perfusion system were carried out on ihs mice. The pancreatic islets were examined histologically, and the mRNA expression of pancreatic β-cell-specific genes or genes associated with monogenic diabetes was examined by RT-qPCR.

Results: The ihs mice showed several distinctive diabetes-related characteristics: (i) the onset of diabetes was observed only in the male mice; (ii) there were no differences in insulin content between the ihs and control mice; (iii) impaired insulin secretion was elicited by glucose, potassium chloride and sulfonylureas; (iv) there was a significant reduction of relative β-cell volume with no signs of inflammation or fibrosis; (v) they showed a normal glycemic response to exogenous insulin; and (vi) the mice were not obese.

Conclusions: The ihs mouse provides a novel murine model of congenital diabetes that shows insulin secretion failure. This model allows not only an analysis of the progression of diabetes, but also the identification of unknown genes involved in insulin secretion.

INTRODUCTION
Type 2 diabetes is a metabolic disorder that is characterized by abnormal glucose homeostasis due to some defect in the secretion and/or action of insulin. There has been a dramatic increase in type 2 diabetes patients in East Asian countries, which now represent one-quarter of the global diabetes population. Although the precise mechanisms that underlie the development and progression of type 2 diabetes have not been fully elucidated, it is thought that a combination of multiple genetic and environmental factors contribute to the pathogenesis of the disease. There has therefore been increased interest in reappraising animal models of type 2 diabetes in which genetic and environmental factors that could influence the development of the disease and related complications can be precisely controlled in vivo to help elucidate the etiology of diabetes and develop new medications. Animal models have made an enormous contribution to the study of diabetes mellitus, providing new information on its management and treatment in humans. Animal models for type 2 diabetes have been widely used for elucidating the genes responsible for the development of type 2 diabetes, the physiological course of the disease and related complications.

Type 2 diabetes in East Asian people is characterized by a lower level of obesity compared with type 2 diabetes in Caucasian, as well as a younger age of onset and β-cell dysfunction. Relatively few people with diabetes in Japan are obese, and the impairment of insulin secretion often develops before the onset of diabetes. Thus, non-obese animal models...
are required to elucidate the complete pathogenesis of diabetes. To this end, we have established a novel, non-obese murine strain with spontaneous diabetes—the insulin hyposecretion (ihs) mouse. The ihs mouse allows not only the analysis of the progression of diabetes, but also the identification of unknown genes involved in insulin secretion. The present study describes the development and characterization of the ihs mouse.

METHODS
Ethical statement
All animal experiments were approved by the President of Kitasato University and National Center for Global Health and Medicine, following consideration by the Institutional Animal Care and Use Committee of Kitasato University (approval ID: no. 17-099) and National Center for Global Health and Medicine (approval ID: no. 17056), and were carried out in accordance with institutional procedures, national guidelines and the relevant national laws on the protection of animals.

Breeding of congenic mice
The ICR-derived glomerulonephritis (ICGN) mouse provides a model of glomerular dysfunction that shows gross morphological changes in the podocyte foot processes that accompany proteinuria (Appendix S1). Previously, we showed that proteinuria in ICGN mice was caused by a deletion mutation in the tensin2 (Tns2) gene (designated Tns2nph)10,11. As the original ICGN mouse is a spontaneous mutant derived from closed colony ICR mice, there is no control strain. We therefore created a control strain by using classical breeding methods to introgress the wild-type Tns2 gene (Tns2WT) from the C57BL/6J (B6) mouse into the inbred ICGN strain (ICGN.B6-Tns2WT)12.

We moved the Tns2WT allele onto the ICGN genetic background by backcrossing for 10 generations, and subsequently homozygous ICGN.B6-Tns2WT/Tns2WT mice were produced by sib mating. During this strain breeding process, we discovered mutant mice with polydipsia, polyuria, hyperglycemia and hypoinsulinemia, which we named ihs mice (Figure 1). As ihs mice are spontaneous mutants derived from ICGN mice, there is no control strain. We therefore created a control strain by using classical breeding methods to introgress the wild-type Tns2 gene (Tns2WT) from the C57BL/6J (B6) mouse into the inbred ICGN strain (ICGN.B6-Tns2WT)12.

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Phenotype measurements
The mice were weighed weekly from 5 weeks-of-age. The nasal-anal length was measured, and the body mass index was calculated at 12, 25 and 52 weeks by taking the weight (g) and dividing it by the square of the nasal-anal length (cm). Body composition was measured by NMR (LF50 BCA-Analyzer; Bruker, Billerica, Massachusetts, USA).

Blood and urine analysis
Urine glucose levels were measured semiquantitatively using Uro-paper III (Eiken, Tokyo, Japan). To obtain paired measurements with urine glucose levels, blood was collected by tail snip immediately after urine collection. The blood glucose levels were measured using Glutest Ace and Glutest Sensor (Sanwa Chemical Co., Tokyo, Japan). Diabetes was diagnosed when the glucose level was >250 mg/dL under ad libitum feeding conditions.

Oral glucose and insulin tolerance tests
The mice were fasted for 16 h, and tail blood glucose was measured at 0, 30, 60, 90 and 120 min after the oral administration of glucose (2 g/kg bodyweight; Otsuka Pharmaceutical, Tokyo, Japan) by gavage. Plasma samples were collected from the retro-orbital venous plexus at 0, 15 and 30 min for insulin measurement. Plasma insulin levels were determined by an ultrasensitive mouse insulin kit (Morinaga Institute of Biological Science Inc., Kanagawa, Japan). The area under the curve (AUC) of insulin secretion was calculated after subtraction of the baseline insulin level at the time the glucose was administered.

An insulin tolerance test was carried out after the mice had fasted for 3 h. Insulin (0.75 unit/kg bodyweight; Humulin, Lilly, Indianapolis, Indiana, USA) was injected into the intraperitoneal space, and blood glucose levels were measured with a glucose monitor at 0, 30, 60, 90 and 120 min after the injection.

Other analyses
Non-fasting plasma insulin and active glucagon-like peptide-1 (GLP-1) levels were measured by enzyme-linked immunosorbent assay (Appendices S2 and S3).
Insulin secretion from in situ perfused pancreata (Appendix S4) and insulin secreted by islets isolated from ihs mice (Appendix S5) were measured.

Pancreata were subjected to histological analysis, including an analysis of islet morphology, as described previously. The details are given in Appendix S6.

RT-qPCR for genes associated with monogenic diabetes was carried out. Details are presented in Appendix S7 and Table S1.

**Statistical analysis**
The results are expressed as mean ± standard error of the mean. Significant differences in AUC or reverse AUC were analyzed using unpaired Student’s t-tests. The results for insulin secretion from the in situ perfused pancreas analysis were analyzed using repeated-measures ANOVA and Student’s t-test. Student’s t-test was used for comparisons of two independent groups, respectively. A P-value <0.05 was considered statistically significant.

**RESULTS**

**Incidence of diabetes, and survival**
The cumulative incidence of diabetes in ihs mice, determined by the casual blood glucose level, is shown in Figure 2a. Diabetes was observed only in male mice. The earliest onset was observed at 9–12 weeks, and its incidence increased with age (33 and 89% at 12 and 42 weeks, respectively). Glucosuria appeared in male mice at 9–12 weeks, concomitant with the onset of diabetes, and was present in 88.9% of mice aged 42 weeks (Figure 2b). Because the onset of diabetes differed between the sexes, we focused our studies on male mice. At 45 weeks-of-age, the survival rate of the male ihs mice was approximately 50%, decreasing to approximately 42% at 51 weeks, compared with 81% survival at 51 weeks in female ihs mice (Figure 2c). This indicated that the onset of diabetes influenced the survival of male ihs mice.

**Clinical features of the ihs mice**
Aged 11 weeks, the ihs mice (32.4 ± 0.4 g, n = 10) were significantly lighter than the ICR mice (33.8 ± 1.9 g, P < 0.01, n = 9). However, at 12 weeks, the ihs mice were heavier than the B6 strain mice (Figure 3a). The bodyweight of the B6 mice continued to increase until 52 weeks-of-age, whereas growth in the ihs mice reached a plateau (35 g) at 25 weeks (Figure 3b), with their weight gradually decreasing to approximately 61% of that of B6 mice at 52 weeks. A significant decrease in body weight was observed after the onset of diabetes. The two strains of mice had similar body mass index values at 25 weeks (Figure 3c), indicating that the ihs mice were non-obese. The analysis of body composition using the NMR method showed that the relative fat percentage was significantly lower in ihs mice, being approximately 47 and 20% of that of B6 mice at 25 and 52 weeks-of-age, respectively, with a concomitant increase in lean mass (Figure 3d). These findings showed that the bodyweight reduction in ihs mice was largely due to decreased fat mass, thought to be associated with the onset of diabetes.

To assess whether the ihs mice were hyperphagic, the daily food intake was monitored in animals fed a standard chow diet ad libitum. At 12 and 25 weeks, the ihs mice consumed approximately 50% more food than the controls, indicating marked hyperphagia (Figure 3e). In addition, a significant increase (P < 0.05) in total water intake was observed in ihs mice throughout the experimental period (Figure 3f). However, no proteinuria was observed and there were no obvious pathological changes in the glomeruli at 45 weeks-of-age in the male ihs mice (data not shown).
Glucose tolerance and insulin response to oral glucose loading and sensitivity to insulin

To characterize the ihs mice in terms of systemic glucose homeostasis and insulin release in vivo, we carried out oral glucose tolerance tests (OGTTs) on ihs and control mice. At 10 weeks-of-age, there was no difference in fasting glucose levels between the ihs and control animals, but the male ihs mice showed typical glucose levels of ≥300 mg/dL, typical of diabetes, at 30–120 min after glucose loading (Figure 4a). Although the female ihs mice showed normal blood glucose levels in the non-fasting state, they showed marked impairment in glucose tolerance (Figure 4b). The glucose AUC values after glucose loading in male and female ihs mice were significantly higher than those in B6 mice. Impaired glucose tolerance was observed in all male and female ihs mice with an exacerbation of glucose tolerance in an age-dependent manner (Figure S1a,b).

Figure 3 Bodyweight, body mass index (BMI), body composition, water consumption and food intake in ihs mice. (a) Change in bodyweight in male ihs (n = 8) and B6 (n = 10). (b,c) Bodyweight in male ihs (n = 4) and B6 mice (n = 5) was measured at the indicated ages. BMI was calculated as bodyweight (g) divided by the square of the anal-nasal length (cm²) at the indicated ages. (d) Comparison of body composition at the indicated ages. (e,f) Water consumption and mean daily food intake were measured at the indicated ages. Data were analyzed by Student’s t-test (**P < 0.01, ihs mice vs age-matched B6).
Insulin concentrations were also measured during the OGTTs. The non-fasting insulin levels (Figure S2) and the plasma insulin levels of the \textit{ihs} and control mice before glucose administration were similar (Figure 4c,d). After glucose loading, the \textit{ihs} mice showed significantly lower insulin levels and the insulin AUC values were also lower (Figure 4c,d). This suggested that \textit{ihs} mice have defective glucose-stimulated insulin secretion at the early phase of diabetes. Insulin release \textit{in vivo} during the OGTTs was markedly impaired in male and female \textit{ihs} mice, resulting in marked impairment in glucose tolerance. The plasma GLP-1 levels after glucose loading in \textit{ihs} mice was comparable with that of the control mice (Figure S3).

To test for insulin sensitivity, we carried out insulin tolerance tests on \textit{ihs} and control mice (Figure 4e,f). The \textit{ihs} mice showed a normal glycemically response to exogenous insulin and were not insulin-resistant.

**Histological examination of pancreatic islets in male \textit{ihs} mice**

Islets from mutant and control mice at 10–12 weeks-of-age were of similar size and shape with a smooth periphery (Figure 5a). No inflammation or fibroblast proliferation was observed in the mutant mouse islets. Immunostaining for insulin showed that insulin-positive cells were regularly distributed. Furthermore, no differences in pancreatic insulin contents were observed between the mutant and control mice (Figure 5b), and the sizes of individual \(\beta\)-cells from the histological sections were similar in the mutant and control mice (Figure 5c). Next, the volume of \(\beta\)-cells relative to the gross volume of the pancreas was determined to analyze sections that were immunostained for insulin. In male \textit{ihs} mice, the relative \(\beta\)-cell volume was significantly lower than that in the control B6 mice (Figure 5b), and the ratio of \(\alpha/\beta\)-cells in the \textit{ihs} islets was significantly increased (Figure 5e). In aged male and female \textit{ihs} mice, the increase in \(\alpha\)-cells was more pronounced (Figures S4 and S5); a marked decrease in the number of islets was observed only in aged male \textit{ihs} mice. In mouse islets under normal conditions, \(\alpha\)-cells are localized in the islet periphery. However, the pattern of peripheral distribution of \(\alpha\)-cells seemed to be disrupted, and some \(\alpha\)-cells were scattered throughout the islet core in the pancreas of \textit{ihs} mice in a manner similar to that observed in diabetes models (Figure 5a)\textsuperscript{12}.

**Insulin secretion from perfused pancreata and isolated islets**

We hypothesized that abnormal insulin secretion was the cause of the hypoinsulinemic hyperglycemia in the \textit{ihs} mice. To test this, we carried out perfusion experiments that examined the time-course of the insulin secretary response to high glucose. In the control pancreata, a change in glucose concentration from 2.8 to 16.5 mmol/L elicited strong peaks, with a 14-fold increase in insulin secretion (Figure 6a). As was expected from the results of the OGTTs, the elevation of glucose concentration from 2.8 to 16.5 mmol/L in the pancreata of the \textit{ihs} mice resulted in a fourfold increase in insulin secretion. In the \textit{ihs} mice, the AUC values of secreted insulin after glucose stimulation (from 10 to 26 min) was significantly impaired compared with that of the control mice (24.5 ± 9 vs 92.4 ± 13.1; \(P < 0.01\); Figure 6b).

Islet \(\beta\)-cell glucose metabolism is accompanied by closure of the adenosine triphosphate-sensitive potassium channels followed by depolarization. We therefore investigated insulin secretion in response to potassium chloride (KCl), a potent insulin secretagogue, to identify which steps in the insulin secretion pathway are impaired in \textit{ihs} mice. The \textit{ihs} pancreata showed a significantly lower increase in insulin secretion compared with the control pancreata (an 8-fold increase vs a 47-fold increase; \(P < 0.05\); Figure 6a), indicating a significant reduction in insulin secretion in the \textit{ihs} mice (Figure 6b). In the batch incubation experiment, the islets isolated from \textit{ihs} mice also showed a significant decrease in insulin secretion compared with those isolated from control mice, when stimulated with glibenclamide, (antidiabetic drug of class sulfonylureas), which causes the \(\beta\)-cell depolarization (Figure S6)\textsuperscript{16}.

These results showed that the insulin secretion capability of \(\beta\)-cells was markedly impaired in \textit{ihs} mice.

**Assessment of pancreatic \(\beta\)-cell gene expression in the islets of \textit{ihs} and control mice**

Glucose-stimulated insulin secretion can be impaired after a reduction in the proteins involved in glucose sensing and insulin secretion. To determine whether the defect in insulin secretion in \textit{ihs} mice was associated with abnormalities in gene transcription, the expression of pancreatic \(\beta\)-cell-specific genes or genes associated with monogenic diabetes was assessed by RT-qPCR (Figure 7). Most of these genes showed no change in expression in comparison with control mice. However, \textit{Glp1r} was significantly upregulated, and the expression levels of \textit{Glis3}, \textit{Glut2}, \textit{Wfs1} and \textit{Epac2} were downregulated, in the islets of the \textit{ihs} mice.

**DISCUSSION**

Animal models of diabetes that more closely recapitulate the features of the human disease would be useful for understanding the pathogenesis and developing new treatment strategies. The \textit{ihs} mice showed several distinctive diabetes-related characteristics: (i) the onset of diabetes was observed only in the male mice; (ii) there were no differences in insulin content between the \textit{ihs} and control mice; (iii) impaired insulin secretion was elicited by glucose and KCl; (iv) there was a significant reduction of relative \(\beta\)-cell volume with no signs of inflammation or fibrosis; (v) they showed a normal glycemically response to exogenous insulin; and (vi) they were non-obese. Nagoya–Shibata–Yasuda (NSY) mice, a type 2 diabetes model derived from ICR mice, showed phenotypes similar to that of \textit{ihs} mice, such as severely impaired glucose tolerance, reduction in glucose-stimulated insulin secretion and differences in diabetes onset based on sex\textsuperscript{17–20}. However, NSY mice showed an increase in the fasting plasma insulin levels, insulin resistance and hypertrophy of pancreatic islets, whereas \textit{ihs} mice had normal fasting plasma
**Glucose**

(a) Blood glucose (mg/dL)

(b) AUC glucose (mg/dL)

(C) Plasma insulin (ng/mL)

(D) AUC insulin (ng/mL)

(E) Blood glucose (% of initial value)

(F) Reverse AUC

**Insulin**

(A) Glucose

(B) AUC glucose

(C) Plasma insulin

(D) AUC insulin

(E) Blood glucose

(F) Reverse AUC

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Figure 4 | Glucose tolerance, insulin secretion and insulin sensitivity in ihs mice. (a,b) Upper figure: oral glucose tolerance test (2 g/kg) at 10–12 weeks-of-age in male B6 (n = 5) and ihs (n = 5) mice, and in female B6 (n = 6) and ihs (n = 5) mice. Blood glucose was measured at 0, 30, 60, 90 and 120 min in response to oral glucose administration in 16 h-fasted mice. Lower figure: the area under the curve (AUC) of the 0–120 min glycemic responses. AUCglucose was calculated using the trapezoidal rule. Data were analyzed by Student’s t-test (**P < 0.01, ihs mice vs pair-fed B6). (c,d) Upper figure: plasma insulin concentrations during the oral glucose tolerance tests (2 g/kg) at 10–12 weeks-of-age in male B6 (n = 5) and ihs (n = 4) mice, and in female B6 (n = 6) and ihs (n = 5) mice. Plasma insulin was measured at 0, 15 and 30 min in response to oral glucose administration in 16 h-fasted mice. Lower figure: AUCinsulin of the 0–30 min % of initial value was calculated using the trapezoidal rule. Data were analyzed by Student’s t-test (**P < 0.01, ihs mice vs age-matched B6). (e,f) Blood glucose (% of initial value) during the insulin tolerance test (0.75 IU/kg) at 10–12 weeks-of-age in male B6 (n = 6) and ihs (n = 8) mice, and in female B6 (n = 7) and ihs (n = 7) mice. The reverse AUC was calculated using the trapezoidal rule. Data were analyzed by Student’s t-test.

Figure 5 | Pancreatic islet morphology. (a) Upper panels: pancreatic histological sections of male B6 and ihs mice at 10–12 weeks-of-age stained with hematoxylin–eosin (HE). Lower panels: immunohistochemistry with antibodies against insulin (green) and glucagon (red). (b) Insulin content, (c) β-cell size, (d) Relative β-cell volume (%) and (e) the ratio of α- to β-cell areas in the pancreas (α/β-ratio). B6 (n = 3) and ihs (n = 3) mice. Data were analyzed by Student’s t-test (*P < 0.05, **P < 0.01). Scale bar, 100 μm.
insulin levels and insulin sensitivity, and showed the relative β-cell volume was lower than that in control mice. Furthermore, the incidence of diabetes in the female NSY mice was 31% at 48 weeks, whereas the female ihs mice did not develop diabetes, although female ihs mice have impaired glucose tolerance. Therefore, we concluded that the ihs mouse was a novel murine model for non-obese type 2 diabetes.

The release of insulin from pancreatic β-cells is regulated by highly complex and sophisticated mechanisms, modulated by many factors, including hormones, neuropeptides and neurotransmitters. With respect to the mechanism in β-cells, we showed that Glut2, Wfs1 and Epac2 expression levels were significantly downregulated (by 50%) in the islets of the ihs mice, and that Glp1r expression was increased threefold. The GLP-1 receptor (GLP1R) is expressed mainly in the alimentary tract, particularly in the pancreatic islet cells, where it mediates the actions of GLP-1 released from the small intestines in response to food intake. GLP-1 is considered to be one of the most important glucose-dependent insulin secretagogues, and is known to cause a rise in intracellular Ca²⁺ concentration. The GLP1R, a G-protein-coupled receptor, is involved in insulin secretion through mechanisms dependent on and independent of PKA. GLP-1 stimulates the expression of glucose transporter 2, which determines the rate of glycolysis and helps to confer the glucose competence to β-cells, thereby increasing the efficacy and potency of glucose as a stimulus for insulin secretion. It has been reported that WFS1 is essential for GLP-1-stimulated cAMP production, and the regulation of insulin biosynthesis and secretion. In pancreatic β-cells, cAMP signaling, which can be activated by various extracellular stimuli,
including hormonal and neural inputs primarily through G-protein-coupled receptors, is vital for the normal regulation of insulin secretion to maintain glucose homeostasis. The activation of cAMP signaling amplifies insulin secretion dependent on exchange protein directly activated by cAMP 2 (EPAC2)27. EPAC2 is essential in the potentiation of insulin granule exocytosis by cAMP, primarily in the first phase of cAMP-potentiated exocytosis, by increasing the number of restless newcomers28. Although the plasma GLP-1 concentration was comparable with the control mouse (Figure S3), the unresponsiveness of the pathway induced by GLP1R activation might cause the upregulation of Glp1r, and downregulation of Glut2, Wfs1 and Epac2 expression in the ihs mouse.

A high concentration of KCl and sulfonylureas can directly cause depolarization of the β-cell plasma membrane, which subsequently triggers an influx of Ca2+ and insulin granule exocytosis16,28. This indicates that the defective insulin release induced by KCl in the perfusion experiments (Figure 6) and by glibenclamide in batch incubation experiments (Figure S6) was not caused by a defect in GLP-1 signaling. The causative gene for insulin hyposcretion in the ihs mouse is not involved in incretin-potentiated insulin secretion. However, the GLP-1 signal elevates cytosolic Ca2+ in β-cells24,29. Thus, the significant upregulation of Glp1r and marked impairment of insulin secretion stimulated by glucose and KCl might be caused by a defect in the Ca2+ signaling pathway in the β-cells of the ihs mouse. Although relative β-cell volume of ihs mice decreased to almost 60% of that of the B6 mouse (Figure 5d), no differences were observed in the insulin content of the pancreata and in the β-cell size between the ihs and control mice (Figure 5b,c), and insulin 1 and 2 mRNA levels were similar in the two strains (Figure 6). This suggests the possibility that more insulin granules are stocked in each β-cell of ihs mice. Therefore, we concluded that the marked dysfunction in insulin secretion observed in the ihs mice at a young age was not caused by a defect in the insulin production or islet architecture and organization, but by a defect in the Ca2+ influx and/or Ca2+ signaling pathway in β-cells.

The incidence of diabetes in the male ihs mice was 90% at 42 weeks-of-age; in contrast, none of the female mice developed diabetes, even though the female ihs mice showed impaired glucose tolerance and the impairment of glucose-induced insulin secretion (Figures 4b,d and S1b). In the present study, we found that the relative β-cell volume in the male ihs mice was lower than that in age-matched B6 mice (Figure 5d), and progressively decreased with aging (Figure S4). In contrast, marked reduction of islets was not observed in aged female ihs mice (Figure S5). It seemed that the reduction of islets in aged male ihs mice was influenced by glucotoxicity. Additionally, glucagon-positive α-cells were evenly distributed along the periphery of the islets in the control mice. However, a relative increase in α-cells along the periphery and in the central core of the islet was more evident with aging in the male and female ihs mice (Figures S4 and S5). A relative increase in α-cells has been reported in many animal models with β-cell deficiency15, as well as in patients with type 2 diabetes30,31. This is thought to result from the collapse of the central β-cell core due to the loss of β-cells rather than from α-cell hyperplasia. A similar sex difference has been observed in a variety of diabetic cases in humans32,33. In addition, many knockout and transgenic models of diabetes show a sex bias34, and the protective effect of estradiol against the development of insulin resistance and diabetes has been widely discussed35,36. We assume that estrogen and prolactin play a protective role in female rodents, as they have a regulatory influence on β-cell function37 and proliferation rates38. Further research is warranted to elucidate the mechanism(s) underlying this sex bias.
Models of spontaneous diabetes are invaluable for the identification of unknown diabetogenic genes. The ihs mouse model involves neither obesity nor insulitis, but is accompanied by notable pancreatic β-cell dysfunction, which distinguishes it from other well-characterized animal models. Although further genetic analysis is required to allow a better understanding of the molecular pathology of ihs mice, our preliminary exome sequencing analysis from ICGN mice and genetic analysis in ihs mice showed no non-synonymous, nonsense or frameshift mutations in the known genes responsible for monogenic diabetes characterized by β-cell failure in humans and rodents. This suggests that the ihs strain can contribute to the discovery of novel causative genes, potentially leading to the development of treatment strategies for hypoinsulinemia and diabetes.

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DISCLOSURE

The authors declare no conflict of interest.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

*Figure S1* | Glucose tolerance test in *ihs* mice at each weeks of age.

*Figure S2* | Non-fasting plasma insulin levels.

*Figure S3* | Plasma active GLP-1 levels after glucose oral administration.

*Figure S4* | Pancreatic islet morphology in aged male *ihs* mice.

*Figure S5* | Pancreatic islet morphology in female *ihs* mice.

*Figure S6* | Insulin secretion in the isolated islets of *ihs* mice.

*Table S1* | RT-qPCR primer sets of genes associated with monogenic diabetes.

*Appendix S1* | Mice.

*Appendix S2* | Non-fasting plasma insulin levels.

*Appendix S3* | Plasma active GLP-1 levels.

*Appendix S4* | Insulin secretion from the in situ perfused pancreas.

*Appendix S5* | Insulin secretion of islets isolated from *ihs* mice.

*Appendix S6* | Islet morphology analysis.

*Appendix S7* | RT-qPCR analysis.