Type 2 Diabetes Mellitus in a Non-Obese Mouse Model
Induced by Meg1/Grb10 Overexpression

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Abstract: We assessed the possibility of C57BL/6-Tg (Meg1/Grb10)isn(Meg1 Tg) mice as a non-obese type 2 diabetes (2DM) animal model. Meg1 Tg mice were born normal, but their weight did not increase as much as normal after weaning and showed about 85% of normal size at 20 weeks of age. Body mass index of Meg1 Tg mice was also smaller than that of control mice. The glucose tolerance test and insulin tolerance test showed that Meg1 Tg mice had reduced ability to normalize the blood glucose level. Blood urea nitrogen (BUN) in Meg1 Tg mice (19.6 ± 1.2 mg/dl) was significantly lower than in controls (22.0 ± 0.8 mg/dl), while plasma triglyceride, insulin, adiponectin, and resistin levels were significantly higher (202.0 ± 23.4 mg/dl vs 146.3 ± 23.4 mg/dl, 152.4 ± 16.3 pg/ml vs 88.1 ± 16.9 pg/ml, 74.4 ± 10.9 µg/ml vs 48.3 ± 7.0 µg/ml, and 4.0 ± 0.2 ng/ml vs 3.6 ± 0.2 ng/ml, respectively). Body, visceral fat weight and liver weights were significantly lower (19.6 ± 0.4 g vs 24.3 ± 0.3 g, 376.7 ± 29.6 mg to 507.5 ± 23.0 mg, and 906.0 ± 41.8 mg to 1,001.0 ± 15.1 mg, respectively). Thus, hyperinsulinemia observed in Meg1 Tg mice indicates that their insulin signaling pathway is somehow inhibited. With high fat diet, the diabetes onset rate of Meg1 Tg mice increased up to 60%. These results suggest that Meg1 Tg mice resemble human 2DM.

Key words: biochemical characterization, Meg1/Grb10 transgenic mouse, non-obese mouse model, type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (2DM) is a life-threatening endocrine disorder that affects as many as 6% to 10% of the population of the world. This type of diabetes is classified as non-insulin dependent diabetes and accounts for higher than 95% of all cases of diabetes [12, 39]. Moreover, recent studies have revealed that the prevalence of 2DM has doubled in the United States over the last 30 years [1].

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Many experimental 2DM model animals have been established from spontaneous mutants and are currently in use in human 2DM research [11, 22, 24, 33, 35]. In spite of much effort, which has focused on using such model animals, some aspects of 2DM remain unclear. To elucidate the pathological character of 2DM, it is necessary to identify which metabolic pathways are responsible for the onset of 2DM. However, in many cases, the relationship between original mutations and their affected metabolic pathways is difficult to explain. Recent progress in molecular biology has enabled us to take another approach to develop novel diabetes model animals through the manipulation of genes that are closely related to glucose metabolism and insulin resistance [6]. Using gene-targeting technology, a number of useful mouse models have recently been developed for the study of the progression of diabetes [4, 17].

Recently, genomic imprinting has been discovered as an important factor in the etiology of 2DM. A number of studies have reported a relationship between genomic imprinting and 2DM in the mouse [13, 21, 30]. Overexpression of some imprinted genes might explain the mechanism of human transient neonatal diabetes [21], and deletion of some imprinted genes has been suggested as the cause of pancreatic β cell dysfunction [13]. Another candidate imprinted gene for 2DM is maternally expressed 1 (Meg1) / growth factor receptor-binding protein (Grb) 10 [23]. Recently, it has been reported that Meg1/Grb10 knockout mice show the embryonic overgrowth phenotype [3]. Meg1/Grb10 transgenic mice Meg1 Tg mice were produced to elucidate Meg1/Grb10 function in vivo [31]. Grb10 interacts with both insulin receptors (IR) and insulin-like growth factor I receptors (IGF-1R) in vitro [8, 9, 27]. Since the IGF-1 signaling pathway is reportedly involved in embryonic growth, Meg1/Grb10 may have a negative effect both on the embryonic growth and postnatal growth phenotypes associated with uniparental duplication of chromosome 11 [7, 23]. Alternatively, Meg1/Grb10 may function in glucose homeostasis, which is regulated by the IR signaling pathway. Insulin binds to IR and activates a signal transduction pathway through its receptor kinase activity. It has been demonstrated that IGF1 functions via IGF1R, while IGF2 functions via both IGF1R and IR, and that each of these signaling pathways contributes to some extent to late embryonic growth [20]. Moreover, mutation studies of human 2DM patients indicate the existence of additional factors in the pathogenesis of this disease [25].

Overall, Meg1 Tg mice have similar character to human 2DM in overexpression of the imprinted Meg1/Grb10 gene that functions negatively for both insulin signaling via IR and IGF-1 signaling via IGF-1R. Therefore, it seems likely that, in the late embryonic stage, when endogenous Meg1/Grb10 expression is very high, Meg1/Grb10 negatively regulates growth via modulation of both the IR and IGF1R cascades [31]. There are few data about biochemical changes in the Meg1 Tg mouse. To be useful in therapeutic research the model mouse has to show a similar phenotype to human 2DM. Furthermore, the incidence rate of the onset of 2DM in Meg1 Tg mice fed on basal diet was reportedly small [30].

In this study, we examined several basic biochemical characters of Meg1 Tg mice as a 2DM model, and the effects of diet on the onset of 2DM. The results indicate that the Meg1 Tg mouse is a useful non-obese 2DM mouse model.

Materials and Methods

Animals

The production and maintenance of Meg1/Grb10 transgenic mice were reported in detail elsewhere [31]. Transgenic (C57BL X C3H) F2 mice were screened by PCR amplification of tail DNA samples using transgene-specific and endogenous Peg1 primer sets. Transgenic-positive founder mice (Meg1 Tg mouse) were backcrossed to C57BL/6NJcl (C57BL/6) mice, and the litters that were used in subsequent studies contained animals that were maintained within the C57BL/6 hybrid background. The Meg1 Tg mouse consists of 4 lines, with names of T10L, T18L, T20L, and T27L. We used these 4 lines for each experiment. Transgenic-negative mice were used as control mice. C57BL/6, NOD/ShiJcl (NOD), KK-Ay/TaJcl (KK-Ay), and BKS.Cg-Leprdb/leprdb/Jcl (BKS) mice were also used in the RT-PCR experiment. Only male mice were used in our experiment.

This study was performed in accordance with the Guidelines for Animal Experimentation of the National...
Institute of Infectious Diseases.

Body weight and body mass index (BMI)

Body weights of all mice used in our experiments were measured weekly from 4 weeks of age to 20 weeks. At 15 weeks of age, mouse length from nose to anus was recorded. Body mass index (BMI) was calculated as body weight (g) / body length^2 (cm).

Food and water intake

The mice were allowed free access to food pellets and water. We used either CMF (Oriental yeast Co., Ltd., Tokyo) or Quick Fat (CLEA Japan, Inc., Tokyo) for normal fat diet (NFD) and high fat diet (HFD), respectively. HFD contains high crude fat and glucose, which gives it a higher calorie count than NFD. Food intake per mouse was calculated as the average of 3 days intake at 11 weeks of age.

Organ weight

At 30 weeks of age 5 Meg1 Tg mice and control mice were sacrificed under deep ether anesthesia and necropsied. Visceral fat and livers were separated and weighed.

Glucose tolerance test and insulin tolerance test

Glucose tolerance tests and insulin tolerance test were performed on 11-week-old Meg1 Tg mice that had been fed on HFD. Glucose tolerance tests and insulin tolerance tests were performed after overnight fasting by administering glucose orally (2.0 g/kg body weight) and 0.3 µl of blood was collected from the tail vein after 0, 30, 60, and 120 min. The blood glucose level was measured by FreeStyle Meter (NIPRO, Osaka). Insulin tolerance tests were performed by an intraperitoneal injection of 1.0 U/kg of human insulin (Eli Lilly Japan, Tokyo) to Meg1 Tg mice and control mice; then 0.3 µl of blood was collected from the tail vein after 0, 30, 60, and 90 min. The blood glucose level was measured by FreeStyle Meter (NIPRO).

Plasma chemistry

Meg1 Tg and control mice were maintained on a normal light/dark cycle. Blood was collected from the mice with heparin at necropsy and inspection. The samples were centrifuged at 13,000 rpm centrifugation, and plasma was collected and stored at –30°C until assay. Plasma leptin, adiponectin, insulin and resistin were assayed by ELISA. Plasma total glucose, total cholesterol (TCHO), ammonia, triglyceride (TG), blood urea nitrogen (BUN), GOT, GPT, ALP, CPK, and LDH were measured using Fuji dry-chem 3000 (FUJIFILM Medical Co., Ltd., Tokyo). Livers and visceral adipose tissues were also recorded.

Histology

For histopathology, pancreas tissue samples were taken from Meg1 Tg and control mice at 20 weeks of age. The tissue samples were fixed in 10% buffer-neutralized formalin solution and embedded in paraffin. Sections were cut at 2 µm thickness and stained with hematoxylin and eosin.

Urinalysis and confirmation of the onset of 2DM

Urine collection was executed by compulsive urination at each weighing time, and urinary glucose was determined by Urolabostick (Biel-Sankyo Co., Ltd., Tokyo). When urinary glucose was detected, blood was collected from the tail vein. Confirmation of the onset of 2DM was decided by the detection of over 300 mg/ml of glucose in blood.

RT-PCR

Total RNA was extracted from livers, pancreata, skeletal muscles, and white and brown adipose tissues from 5 mice each of the Meg1 Tg, NOD, KK-A^y, BKS, and C57BL/6 strains of mice at 11 weeks of age, using the RNeasy system (QIAGEN K. K., Tokyo) according to the manufacturer’s instructions. For the RT-PCR analysis, cDNA was synthesized from 1 µg of the total RNA using the SuperScript III First-Strand cDNA Synthesis System (Invitrogen Japan K. K., Tokyo) according to the manufacturer’s instructions. The cDNA was PCR-amplified in 50 µl of reaction mixture containing 25 µl of TaqMan master mix (Applied Biosystems Japan Ltd., Tokyo) and 500 nM of the gene-specific (Meg1/Grb10, uncoupling protein 1 (Ucp1), glucose transporter 4 (Glut4), and G3PDH for normalization) TaqManProbe. The assays were performed in triplicate and the copy number of the Meg1/Grb10, Ucp1, and Glut4 RNA were
calculated with an ABI Prism 7900 Sequence Detector (Applied Biosystems Japan). The data for each tissue were normalized to an internal standard (G3PDH).

Statistical analysis

Measurement data are shown as the mean value ± standard error (Mean ± SE). Statistical analysis of the data was performed using a one-factor ANOVA followed by Student’s t-test. Comparison of the mean was calculated by Bonferroni’s method. Covariance analysis was performed by Levene’s method. Calculation of confidence limits and significance testing were made at a level of \( P=0.05 \).

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Results

Postnatal growth curve

Fig. 1 shows the body weights of Meg1 Tg mice and controls that were fed either normal fat diet (NFD) or high fat diet (HFD). The weight of Meg1 Tg mice was normal until 4 weeks of age under both NFD and HFD diet conditions. However, their body weights did not increase as much as control mice after weaning and were 12 to 15% smaller than those of control mice at 20 weeks of age. These differences were statistically significant \( (P<0.05) \).

Food intake and BMI

For average food intake, no differences were observed between Meg1 Tg mice and controls under both diet conditions at 11 weeks of age (Fig. 2A: fed with HFD, 1.74 ± 0.02 g/day/10 g of body weight vs 1.65 ± 0.03 g/day/10 g of body weight; and with NFD, 1.65 ± 0.02 g/day/10 g of body weight vs 1.51 ± 0.07 g/day/10 g of body weight). However, BMI of Meg1 Tg mice were significantly lower than those of control mouse fed with HFD \( (P<0.05) \) both at 15 and 30 weeks of age (Fig. 2B: BMI at 15 weeks of age, 0.27 ± 0.01 g/cm\(^2\) vs 0.32 ± 0.03 g/cm\(^2\); and at 30 weeks of age, 0.29 ± 0.05 g/cm\(^2\) vs 0.36 ± 0.07 g/cm\(^2\)).

Glucose tolerance test and insulin tolerance test

The plasma glucose level of Meg1 Tg mice fed with HFD at 11 weeks of age was significantly higher than those in both Meg1 Tg mice and controls fed with NFD \( (P<0.05) \), indicating that their glucose tolerance was reduced (Fig. 3A). The reduction in blood glucose concentration after intraperitoneal administration of insulin was significantly delayed in Meg1 Tg mice compared to control mice fed with either HFD or NFD \( (P<0.05) \), indicating that Meg1 Tg mice had insulin resistance (Fig. 3B).

Organ weight

The weights of body, visceral fat, and liver, and the visceral fat/body weight ratios and liver/body weight ratios of Meg1 Tg and control mice at 10 to 12 weeks of age are shown in Table 1. The effect on organ weight by HFD feeding in Meg1 Tg mice was examined. When mice were fed HFD, weights of body, visceral fat and livers of Meg1 Tg mice were significantly lower than the controls \( (P<0.05) \), while liver/body weight ratio of Meg1 Tg mice was significantly higher than that of control mice \( (P<0.05) \). When Meg1 Tg mice were fed NFD, their body weight was significantly lower than the controls \( (P<0.05) \). There were no differences in visceral fat/body weight and liver/body weight ratios between Meg1 Tg and control mice irrespective of diet.

Plasma chemistry

The data on BUN, TG, insulin, adiponectin, resistin,
Fig. 2. Daily intake and BMI change in control and Meg1 Tg mice. (A) Daily intake of Meg1 Tg (■) and control (□) mice at 11 weeks of age that were fed with NFD or HFD. There were no significant differences between the diet groups. (B) BMI of Meg1 Tg and control mice fed with HFD were calculated at 15 and 30 weeks of age. BMI of Meg1 Tg mice (■) at both ages were significantly lower than those of control mice (□) (*P<0.05).

Fig. 3. Glucose and insulin tolerance level of Meg1 Tg and control mice. (A) Glucose tolerance in mice at 11 weeks of age that had been fasted overnight (n=10). (B) Insulin tolerance in mice at 11 weeks of age that had been fasted overnight (n=10). Plotted values are means ± SE for 10 mice per group. Open square (□), control mice fed NFD; filled square (■), control mice fed HFD; open circle (○), Meg1 Tg mice fed NFD; filled circle (●), Meg1 Tg mice fed HFD. *P<0.05 (Student’s t-test). Glucose levels and their reduction rate in Meg1 Tg mice were significantly higher than in control mice.
IGF-1, leptin, ammonium, and glucose, measured at 10 to 12 weeks of age, are shown in Table 2. Irrespective of diet, plasma BUN in Meg1 Tg mice was significantly lower than in control mice (P<0.05). Plasma TG, insulin adiponectin and resistin in Meg1 Tg mice were significantly higher than in control mice (P<0.05), whereas plasma IGF-1 of Meg1 Tg mice tended to be lower than in controls. When mice were fed NFD, the plasma leptin level of Meg1 Tg mice was significantly higher than that of control mice. For, mice fed HFD, the plasma leptin level of Meg1 Tg mice was almost the same as the value of control mice (Table 2). Plasma glucose and ammonia in both NFD- and HFD-fed Meg1 Tg mice tended to be higher than those of control mice.

Other measured biochemical markers such as TCHO, GOT, GPT, ALP, CPK, and LDH plasma concentrations measured in Meg1 Tg and control mice of the same age were almost similar (data not shown).

**Histological analysis**

We found two histological abnormalities in the pancreatic tissues of Meg1 Tg mice fed NFD: atrophy of the pancreatic acinus cells and an increase in adipocytes at 20 weeks of age; and enlargement of islet of Langerhans (Fig. 4B). When Meg1 Tg mice were fed HFD, the above-noted pathological abnormalities were more severe than these of NFD feeding (Fig. 4C). Pathological abnormalities did not develop in control mice fed NFD or HFD (Fig. 4A).

**The onset rate of type 2 diabetes**

The onset rates of 2DM between Meg1 Tg mouse fed NFD and HFD at 25 weeks were 11.3% and 60.0%, respectively (Fig. 5A). This clearly demonstrates that feeding with HFD induces 2DM in Meg1 Tg mice. There were no symptoms of 2DM in control mice up to 30 weeks of age.

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**Table 1.** Liver, fat, and body weights of Meg1Tg and control mice at 10 to 12 weeks of age

<table>
<thead>
<tr>
<th></th>
<th>Control (NFD)</th>
<th>Meg1 Tg (NFD)</th>
<th>Control (HFD)</th>
<th>Meg1 Tg (HFD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>20.8 ± 0.6</td>
<td>18.4 ± 0.3a</td>
<td>24.3 ± 0.3</td>
<td>19.6 ± 0.4a</td>
</tr>
<tr>
<td>Visceral fat weight (mg)</td>
<td>197.6 ± 46.0</td>
<td>187.0 ± 68.6</td>
<td>507.5 ± 23.0</td>
<td>376.7 ± 29.6ab</td>
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<tr>
<td>Visceral fat/body weight ratio (mg/g)</td>
<td>9.0 ± 0.9</td>
<td>8.9 ± 1.6</td>
<td>18.8 ± 2.6</td>
<td>20.0 ± 1.1</td>
</tr>
<tr>
<td>Liver weight (mg)</td>
<td>900.2 ± 30</td>
<td>977.4 ± 38.5</td>
<td>1,001.0 ± 15.1</td>
<td>906.0 ± 41.8a</td>
</tr>
<tr>
<td>Liver/body weight ratio (mg/g)</td>
<td>44.5 ± 3.7</td>
<td>51.6 ± 4.9</td>
<td>41.2 ± 1.3</td>
<td>44.9 ± 2.1b</td>
</tr>
</tbody>
</table>

Values are the means ± SE for 10 mice per control and 10 mice per Meg1Tg. Meg1Tg (HFD) and control (HFD) mice were fed HFD. Meg1Tg (NFD) and control (NFD) mice were fed NFD. aP<0.05 vs control, bP<0.05 vs Meg1 Tg (NFD).

**Table 2.** Plasma chemistry in Meg1 Tg and control mice at 10 to 12 weeks of age

<table>
<thead>
<tr>
<th></th>
<th>Control (NFD)</th>
<th>Meg1 Tg (NFD)</th>
<th>Control (HFD)</th>
<th>Meg1 Tg (HFD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>26.7 ± 1.9</td>
<td>20.6 ± 0.7</td>
<td>22.0 ± 0.8</td>
<td>19.3 ± 1.2a</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>69.3 ± 23.2</td>
<td>90.6 ± 21.9</td>
<td>146.3 ± 11.4</td>
<td>202.0 ± 23.4ab</td>
</tr>
<tr>
<td>Insulin (pg/ml)</td>
<td>92.5 ± 13.4</td>
<td>153.3 ± 14.3</td>
<td>88.1 ± 16.9</td>
<td>152.4 ± 16.3a</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>23 ± 4.01</td>
<td>46.8 ± 3.2</td>
<td>48.3 ± 7.0</td>
<td>74.4 ± 10.9ab</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>2.28 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.6 ± 0.2</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>395.8 ± 43.1</td>
<td>232.5 ± 28.3</td>
<td>358 ± 49.5</td>
<td>272.1 ± 24.4</td>
</tr>
<tr>
<td>Leptin (pg/ml)</td>
<td>548 ± 171</td>
<td>1,136.0 ± 267.0</td>
<td>1,036.0 ± 161.0</td>
<td>1,008.0 ± 146.0</td>
</tr>
<tr>
<td>NH3 (μg/dl)</td>
<td>143.1 ± 24.2</td>
<td>162.0 ± 12.2</td>
<td>223.1 ± 32.6</td>
<td>280.7 ± 50.9ab</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>161 ± 16.9</td>
<td>163.0 ± 12.6</td>
<td>172.8 ± 9.4</td>
<td>199.7 ± 58.4</td>
</tr>
</tbody>
</table>

Values are the means ± SE for 10 mice per control and 10 mice per Meg1Tg. Meg1Tg (HFD) and control (HFD) mice were fed HFD. Meg1Tg (NFD) and control (NFD) mice were fed NFD. aP<0.05 vs control, bP<0.05 vs Meg1Tg(NFD).
Meg1/Grb10, Ucp1, and Glut4 expression in Meg1 Tg and 3 diabetes model mice

For further analytical research, we examined diabetes related gene expressions of Meg1 Tg mice in comparison with NOD, KK-A<sup>+</sup>, BKS, and C57BL/6 mice. Meg1/Grb10, Ucp1, and Glut4 expression ratios against control gene are shown in Fig. 6. Meg1/Grb10 gene expression of skeletal muscle of Meg1 Tg mice was 100 times higher than those of the other 3 diabetes model mice and the C57BL/6 mouse (Fig. 6A: *P* < 0.05). In contrast, Glut4 expression of skeletal muscle of Meg1 Tg mice was significantly lower than these of the 3 diabetes model mice and the C57BL/6 mouse (Fig. 6C: *P* < 0.05). There were no differences of Ucp1 gene expression in brown adipose tissue between Meg1 Tg and the 3 diabetes model mice and the C57BL/6 mouse (Fig. 6C: *P* < 0.05). Meg1 Tg mice fed HFD showed suppression of Ucp1 gene expression in brown adipose tissue (Fig. 6).

Discussion

We evaluated the Meg1 Tg mouse as a non-obese 2DM animal model in this study. Two of the major defects seen in 2DM are insulin resistance of targets, such as liver, muscle and adipose tissues, and impaired insulin secretion from pancreatic β-cells [26, 37, 38]. Histological analysis revealed the pancreatic abnormality in Meg1 Tg mice (Fig. 4). Moreover, Meg1 Tg mice showed both the insulin resistance and glucose intoler-
ance (Fig. 3). The plasma insulin concentration of Meg1 Tg mice was significantly higher than in control mouse (Table 2). These data demonstrate that the dysregulation of plasma glucose was caused by insulin resistance. Our results provide support for Meg1 Tg mouse as a 2DM mouse model. Moreover, the body weights of Meg1 Tg neonates were slightly lower than controls, and this difference increased with growth up to 12 to 15% of body weight. BMI of Meg1 Tg mice was also smaller than control mice (Figs. 1 and 2). Body, visceral fat weight and liver weights were also slightly lower in Meg1 Tg mice than in control mice. However, the visceral fat/body weight and liver/body weight ratios of Meg1 Tg mice were similar to those of control mice (Table 1). Overall, the Meg1 Tg mouse showed a non-obese mouse character.

There are several spontaneous polygenic models of 2DM, such as the OLETF rat, the KK-A' mouse, the NSY mouse, and the BKS mouse, which develop overt obesity and hyperinsulinemia prior to the onset of diabetes [7, 14, 34]. The KK-A' mouse and the BKS mouse showed obesity compared with controls [33, 35]. However, the Meg1 Tg mouse model showed a non-obese character. Only limited data exist for 2DM in Japanese subjects, probably because Japanese people are relatively lean. Some Japanese 2DM patients are non-obese patients and the causal factors of 2DM onset is being analyze by many laboratories [15, 28]. The Meg1 Tg diabetes mouse model may provide a useful tool for these researches.

Biochemical analysis data of the Meg1 Tg mouse demonstrated metabolic abnormality. Plasma BUN in
Meg1 Tg mice was significantly lower than in control mice. Plasma TG, insulin, adiponectin, and resistin levels of HFD-fed Meg1 Tg mice were significantly higher than those of control mice. The mean concentration of TG in Meg1 Tg mice, especially with HFD feeding, was over 200 mg/dl, a condition that is called high-fat plasma. High-fat plasma is said to be a precursor of diabetes [19]. A high level of plasma adiponectin and low BMI were found in Meg1 Tg mice fed HFD (Fig. 2B). There was an inverse correlation between plasma adiponectin and BMI in the Meg1 Tg mouse (data not shown). This shows that the Meg1 Tg mouse has characteristics similar to human 2DM. The circulating IGF-1 level in Meg1 Tg mice tended to be lower than that of control mice (Table 2). A lower IGF-1 level could explain the lower body weight of the Meg1 Tg mouse due to the role of IGF-1 signaling in postnatal growth [10]. Overall, the biochemical data suggest that HFD feeding in the Meg1 Tg mouse induces human 2DM related characteristics. In a comparison of the biochemical data between the Meg1 Tg mouse and other 2DM model mice, such as KK-A<sup>1</sup> and BKS mouse, the Meg1 Tg mouse did not show plasma insulin and a remarkable rise of plasma glucose levels like KK-A<sup>1</sup> and BKS mice, but showed a property that is characteristic of human 2DM, a rise of adiponectin level [5, 16, 18]. In addition, it is a very unique characteristic that this property is caused by environmental factors such as diet.

In mRNA expression analysis, diabetes related genes, such as Grb10, Ucp1, and Glut4, in the Meg1 Tg mouse were compared with 3 famous diabetes mouse models (Fig. 6). Regarding the relationship between the Meg1/Grb10 gene and the non-obese character of the Meg1 Tg mouse, overexpression of the Meg1/Grb10 gene may hold the IGF-1 acceptor signal transduction system in check during development in the embryonic stage and the holding effect would be maintained until long after birth [31]. It is reported that maternal duplication of proximal chromosome 11 retards embryonic growth, whereas paternal duplication promotes growth [2]. A recent Grb10 knockout study has clearly demonstrated that Meg1/Grb10 is the gene responsible for embryonic overgrowth observed in paternal duplication of the chromosome 11 region [3], although the effect of Meg1/Grb10 overproduction remains to be addressed. Our data may provide answers to these puzzling issues. Grb10 regulates the insulin signaling and sensitivity in vivo [36]. This suggests that a high level of the Grb10 gene could affect insulin resistance in the Meg1 Tg mouse. The reduction in intracellular lipid by constitutive expression of Ucp1 reflects down-regulation of fat synthesis rather than up-regulation of fatty acid oxidation [32]. The down-regulation of Ucp1 in Meg1 Tg mice fed HFD could explain the non-obese character of the Meg1 Tg mouse. The quantity of expression of Glut4 mRNA in skeletal muscle decreased significantly in Meg1 Tg mice (Fig. 6C). GLUT4 translocation can be activated by insulin, to which the Meg1 Tg mouse has resistance, and may be related to the GLUT4 decrease in skeletal muscle.

Recently, association of hGrb10 genetic variations with 2DM in Caucasian subjects has been reported [2]. There is no evidence of such a correlation in Japanese subjects. However, it has been revealed that the expression of hGrb10 was influenced by epigenetic alterations. It is important to investigate hGrb10 expression and the relationship to environmental conditions including diet in 2DM model mice.

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