Neuroendocrinology Letters Volume 42 No. 3 2021
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Establishment of a diabetes susceptible family in the Bama minipig (BMP) by N-ethyl-N-nitrosourea (ENU) induction

Xiaohua Su[1#], Zhidong Li[1#], Bayaer Nashun[1], Juncheng Deng[2], Min Yue[2], Ming Lv[3], Weiwang Gu[2]

1 Laboratory Animal Center, Guangdong Medical University, Dongguan, Guangdong, 523808, China
2 Laboratory Animal Center, Southern Medical University, Guangzhou, Guangdong, 510515, China
3 Dongguan Songshan Lake Pearl Laboratory Animal Science and Technology Co., Ltd., Guangdong, 523808, China
# These authors are the first authors.

Correspondence to: *Main Corresponding Author*: Xiaohua Su,
Guangdong Medical University, Dongguan, Guangdong, 523808, China.
e-mail: xiaohausu08@126.com.
Co-Corresponding Author: Weiwang Gu.
tel.: +86-15217506675; fax: +86-21-57643271; e-mail: weiwanggu123@163.com

Submitted: 2020-05-07 *Accepted: 2020-09-10* *Published online: 2020-12-10*

Key words: **Bama minipig; diabetes; animal model**

Neuroendocrinol Lett 2021; **42**(3):177–184 **PMID:** 34279861 NEL420321A01

Abstract

BACKGROUND: Diabetes mellitus (DM), characterized by hyperglycemia, is one of the leading causes of death worldwide. An appropriate DM animal model to explore the underlying mechanism of DM and develop new antidiabetic drugs is still desirable. Here, we aim to provide alternatives of DM animal models for medical researches.
OBJECTIVE: To establish a diabetes susceptible family in the Bama minipig (BMP) by N-ethyl-N-nitrosourea (ENU) induction.
METHODS: Male BMPs with hyperglycemia were selected from G1 and bred by the inbreeding strategy. After 5 generations, parameters such as fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG), intravenous glucose-tolerance test (IVGTT), and insulin resistance were determined to evaluate susceptible family members.
RESULTS: The male BMP 2907 (FPG = 6.1 mmol/L, IGVTT 2hPG = 11.9 mmol/L) with hyperglycemia was selected from G1 to generate the 2907 hyperglycemic family. With the number of breeding generations, average FPG levels in BMPs increased significantly ($p < 0.05$). G5 displayed the characteristics of elevated FPG, insulin resistance, dyslipidemia and abnormal glucose tolerance ($p < 0.05$).
CONCLUSION: A diabetes susceptible family has been successfully established, which might be used for further inbreeding or induced to mimic the phenotype of diabetes.

INTRODUCTION

Diabetes mellitus (DM), the seventh leading cause of death in the USA as well as worldwide, is increasingly considered a major non-communicable disease threatening both affluent and non-affluent societies (Narayan *et al.* 2000; Glovaci *et al.* 2019). The latest IDF Diabetes Atlas report showed that about 425 million adults suffered from diabetes in 2017 worldwide. It is estimated that 629 million individuals will suffer from diabetes by 2045. Therefore, DM is a severe health problem that

imposes a heavy burden on individuals and the society at large. Diabetes prevention and treatment has therefore become a hot topic for scholars around the world (Cho *et al.* 2018). Under the circumstances, it is very important to establish an appropriate animal model of diabetes to explore the underlying mechanism of diabetes and develop novel antidiabetic drugs. At present, animal models of diabetes are mainly established by surgical excision of part or all of the pancreas, high glucose and high fat diet and/or induction with streptozotocin (STZ), the transgenic/gene knockout method, and the genetic breeding and selection method (Acharjee *et al.* 2013; Strauss *et al.* 2012; Yan *et al.* 2018).

Rodent models have long been a major tool for diabetes research because of low maintenance costs, well-studied genomics and ease of genetic engineering. However, due to clear metabolic and physiological differences between humans and rodents, research progress has been undoubtedly hampered with confusing biomedical discoveries generated, which hinders the clinical translation of potential prevention and intervention tools for diabetes (Acharjee *et al.* 2013; Aigner *et al.* 2008). Compared with non-human primates and canines, miniature pigs or minipigs are less subject to resource and ethical constraints. Due to the high similarity between pigs and humans in terms of physiology, anatomy, blood biochemical indexes, diet and drug metabolism, in addition to the availability of pig's whole genome sequencing data, the miniature pig appears to be the best supplement and expansion, which can be used in addition to rodent diabetes models (Bassols *et al.* 2014; Renner *et al.* 2018; Spurlock & Gabler 2008).

At present, many scholars have attempted to establish a suitable minipig model by using the above methods. For example, Strauss A *et al.* compared two minipig diabetes models by total pancreatectomy and STZ induction, respectively. Although both methods reliably induced diabetes in GMP, the PE-GMP group clearly had more health problems and required more time and resources. The PE-GMP model, however, was better in eliminating endogenous insulin and C-peptide compared with the STZ-GMP mode (Strauss *et al.* 2012). DM model establishment by STZ leads to direct injury of islet beta cells, which is often used in type 1 DM modeling (Wu & Yan 2015). Due to islet beta cell regeneration leading to phenotype instability, STZ induction modeling is not suitable for long term experiments (Strauss *et al.* 2012). High glucose and high fat diet could also produce diabetes symptoms. However, at least 5-10 months of diet treatment should be applied (Yuan & Tao 2016). DM is a polygenic disease, and simple gene knockout has great limitations. In addition, this method is very difficult to perform, and the problem of generation after successful model establishment remains (Yuan & Tao 2016; Perleberg *et al.* 2018; Wolf *et al.* 2014). In the genetic breeding method, the first step involves obtaining spontaneous diabetic boars. The difficulty here is that experimental minipigs often tolerate diabetic factors such as lack of exercise and excess energy due to their origin from wildtype swines, and spontaneous diabetic boars are rarely found (Yuan & Tao 2016). Overall, the establishment of minipig diabetic animal models remains to be explored and improved.

N-ethyl-N-nitrosourea (ENU)-induced mutagenesis is an effective forward genetic approach for identifying functional genes and generating animal models. ENU is a potent mutagen that primarily induces point mutations and chromosome rearrangements in the genome in a random manner (Schimenti & Bucan 1998). Bernhard Aigner *et al.* successfully induced murine diabetic models by ENU [7]. The present study aimed to use Bama minipigs to generate large mammal gene mutation and animal models of the human disease by ENU mutagenesis screening. In a previous work, we have generated minipigs with traits simulating human Waardenburg Syndrome, Mondini dysplasia, Oculocutaneous Albinism II, Congenital Hypothyroidism, and Harlequin Ichthyosis (Hai *et al.* 2017). In the latest screening, a boar with FPG ≥ 6.1 mmol/L was obtained, versus averaged FPG levels of 2.0-3.5 mmol/L in minipigs raised with the same environment. It is promising to utilize this line to generate diabetes susceptible family, which would result in the establishment of spontaneous DM models in minipigs.

MATERIALS AND METHODS

Experimental animals

Bama minipigs were obtained from Dongguan Songshan Lake Mingzhu experimental animal science and technology Co., Ltd. The experimental animal production license number was SCXK (Guangdong) 2012-0030, and the experimental animal license number was SYXK (Guangdong) 2012-0123. The experiments were approved by the welfare and ethics committee and completed in general large animal laboratory of Dongguan Songshan Lake Pearl Laboratory Animal Science and Technology Co., Ltd. The minipigs were fed twice daily at 8-9 AM and 4-5 PM according to the standard of 4% body weight with unlimited water supply.

Reagents

Porcine insulin ELISA Kit (CSB-E06828p), porcine glucagon ELISA Kit (CSB-E04854p) and porcine HbA1c ELISA Kit (CSB-E17546p) were from Wuhan Huamei Bioengineering Co., Ltd., with reagent sensitivity of 1 μIU/ml. 50% Glucose Injection (Tiangen biochemical technology (Beijing) Co., Ltd.) was also used.

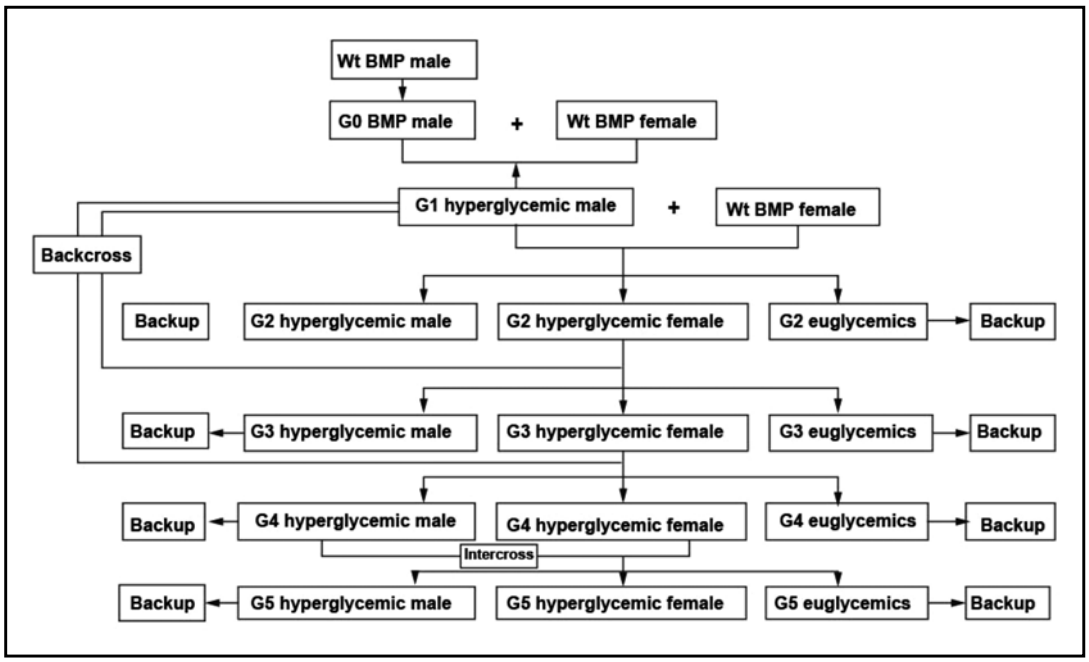

Fig. 1. The detailed breeding steps

Instruments and equipment

A blood glucose meter (Ultraeasy, J&J), blood glucose test papers (Ultraeasy, J&J), a low temperature high-speed centrifuge (Eppendorf 5810 R), a microplate spectrophotometer (Thermo FC), blood analyzer (Sysmex XT-1800 i), an automatic biochemical analyzer (OLYMPUS AU 2700), a desktop centrifuge (Eppendorf 5418) and a water bath (Thermo Fisher) were employed.

Selection of a male BMP and subsequent breeding strategy

After ENU induction, the body weight, blood glucose, physiological and biochemical indexes of G1 were tested once per month. The male BMP with highest blood sugar levels was selected for subsequent breeding. The detailed breeding steps are described in Figure 1.

Grouping and index determination

The experiments were divided into four steps. (1) Male BMPs vs. controls: fasting blood glucose (FPG) was measured once a month starting at 13 months old to the end of the experiment, with glucose tolerance assessed at the end of the experiment. (2) G3 generation hyperglycemic BMPs vs. controls; there were 6 animals in all, including 3 males and 3 females in each group, with FPG measured at 6 months old. (3) G4 generation hyperglycemic BMPs vs. controls; there were 6 animals, including 3 males and 3 females in each group, with FPG assessed at 6 months old. (4) G5 generation hyperglycemic BMPs vs. controls; there were 6 animals, including 3 males and 3 females in each group, with FPG, 2-hour plasma glucose (2hPG), insulin (INS), glucagon (GC), glycosylated hemoglobin A1c (HbA1c), homeostasis model assessment for insulin resistance (HOMA-IR), blood physiological and biochemical indexes, and intravenous glucose tolerance (IVGTT) assessed at 6-month-old.

FPG and 2hPG measurements

Fresh blood was collected from the anterior vena cava plexus, and FPG and 2hPG were assessed with a J&J blood glucose meter. For 2hPG measurement, blood was drawn exactly 2 hours after food intake.

IVGTT test

50% Glucose Injection was administered through the ear vein at a dose of 1.2 ml/kg within 4 minutes (Chen *et al.* 2009). Blood samples were collected from the anterior vena cava plexus for blood glucose measurements at 0, 10, 30, 60 and 120 minutes after injection, respectively.

Glycometabolism related indicators

Blood samples were centrifuged, and serum was used for the detection of glycometabolism indexes. ELISA was used to detect INS, GC and HbA1c.

Insulin resistance index (HOMA-IR)

The HOMA-IR was determined according to fasting blood glucose and fasting insulin as follows. Insulin resistance index (HOMA-IR) = fasting blood glucose * fasting insulin / 22.5 (Matthews *et al.* 1985).

Assessment of physiological and biochemical indexes

Fasting blood was collected from the anterior vena cava plexus and aliquoted into EDTA-K2 tubes (2 ml/tube) and non-anticoagulant tubes (5 ml/tube) for physiological and biochemical measurements, respectively. For biochemical measurements, 5 ml blood in

Tab. 1. Body weight, FPG, 2hPG and glucose metabolism related indexes

	Control (n=6)	Hyperglycemia (n=6)
Body weight (kg)	18.78±1.58	19.01±0.72
FPG (mmol/L)	3.15±0.69	6.53±1.76**
2hPG (mmol/L)	3.37±0.58	7.08±1.05**
INS (μIU/mL)	17.45±1.39	14.66±2.98
GC (pg/mL)	291.76±28.57	273.25±40.03
HbA1c (ng/mL)	236.07±50.48	300.56±32.39*
HOMA-IR	2.43±0.48	4.15±1.19**

*$P < 0.05$ and **$P < 0.01$, compared to the normal group.

non-anticoagulant tubes was centrifuged at 2500g for 5 min for serum preparation. Then, while blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), red blood cell specific volume (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), red blood cell distribution width (RDW-SD and RDW-CV), neutrophil (NEUT), lymphocyte (LYMPH), monocyte (MONO), eosinophil (EO), and basophil (BASO) levels were assessed. Blood biochemical indexes were measured, including total protein (TP), albumin (ALB), globulin (GLO), indirect bilirubin (IBIL), direct bilirubin (DBIL), total bilirubin (TBIL), cholesterol (CHOL), low density lipoprotein cholesterol (LDL-CH), high density lipoprotein cholesterol (HDL-CH), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK) and lactate dehydrogenase (LDH) amounts.

Statistical analysis

Data are shown as mean ± standard deviation (SD). P<0.05 was considered statistically significant. Data analysis was performed with the SPSS software (SPSS, USA).

RESULTS

Male BMP selection and breeding

Hyperglycemia is the most prominent feature of DM, and is mainly used as a diagnostic indicator of DM clinically. According to the diagnostic criteria of the American Diabetes Association, diabetes can be diagnosed with one of the following conditions: (1) FPG ≥7.0 mmol/L; (2) oral glucose tolerance test 2hPG ≥11.1 mmol/L; (3) glycosylated hemoglobin HbA1c ≥6.5%; (4) typical diabetic symptoms and blood glucose PG ≥ 11.1 mmol/L at any time (the first three need to be re-examined for definite diagnosis). After 5 generations of breeding, a total of 66 families (3800 pigs) were established. The BMP G1 male with highest blood glucose levels was selected, numbered 2907, and used to establish the 2907 family line (Figure 2). After long-term monitoring, FPG levels in the male BMP 2907 varied in the range of 6.1-9.9 mmol/L (see Figure 2), while the FPG levels of other control BMPs in the same environment were 2.0-3.5 mmol/L. The male BMP 2907 was used to mate a normal BMP female, and progenies with FPG above 5.0 mmol/L were selected for subsequent breeding (Yan *et al.* 2018). After 5 generations of breeding, male BMP 2907 displayed normal body weight, good physical condition, exuberant saliva secretion and normal sexual behavior, except for

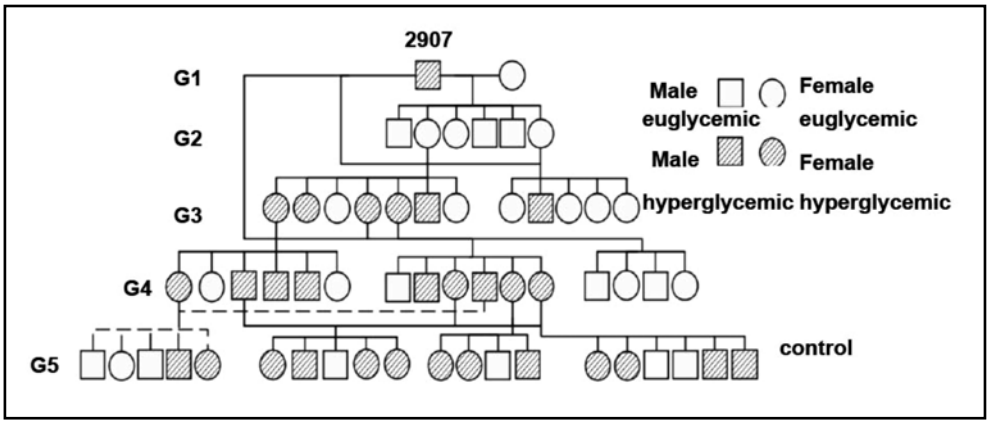

Fig. 2. The family tree of 2907

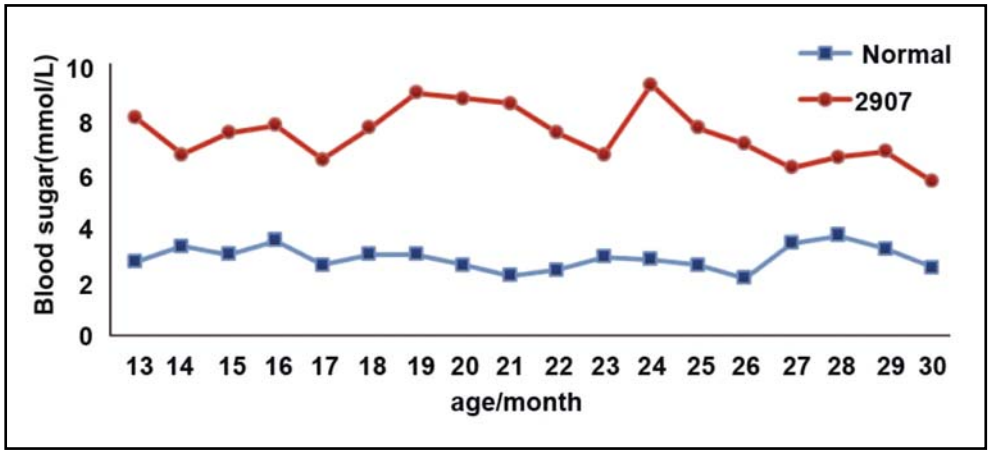

Fig. 3. Fasting blood-glucose of 2907 for 25 months

hyperglycemia (abnormal FPG and 2hPG of IVGTT of 11.9 mmol/L, Table 1). According to the current physical condition, male BMP 2907 could be continually used for 2 years or more.

Hyperglycemic phenotypes in G3, G4 and G5 of 2907 family

The inherited hyperglycemia in the 2907 family tree began in the G3 generation, and the numbers of individuals with increased blood glucose were high in the G3, G4 and G5 generations. Hyperglycemic BMPs in Figure 3 showed FPG levels at 6 months of age: G3 generation, 5.93±0.54 mmol/L; G4 generation, 6.75±0.70 mmol/L; G5 generation, 7.48±0.85 mmol/L. Compared with the G3 generation, both G4 and G 5 generations showed significantly increased FPG levels ($p < 0.05$). The characteristics of hyperglycemia in the G5 generation were more obvious, with more than 50% individuals having FPG levels above 6.0 mmol/L.

IVGTT

After 50% glucose injection, blood glucose levels in both groups immediately increased to 28 mmol/L, and then gradually recovered. At 60 min, the control group basically showed fasting blood glucose level, while the hyperglycemia group showed a drop to 3.78±0.93 mmol. At 120 min, blood glucose levels in the hyperglycemia group returned to 5.77±1.07 mmol/L. The hyperglycemia group showed significantly higher FPG, 30-min glucose levels and 120-min glucose levels compared with the control group ($p < 0.05$); however, there were no significant differences in 0-minute, 10-minute and 60-minute blood glucose levels during the IVGTT between the two groups (Figure 4).

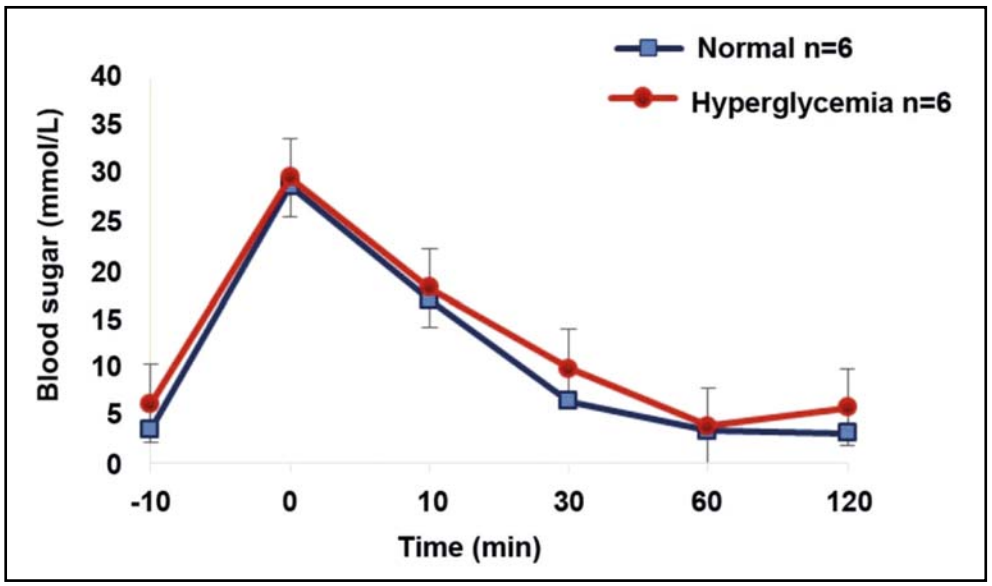

Fig. 4. Intravenous glucose tolerance test results. *$P < 0.05$ and **$P < 0.01$, compared to the normal group.

Tab. 2. Blood physiological indexes

	Normal (n=6)	Hyperglycemia (n=6)
WBC (10^9/L)	20.16±3.15	24.06±9.46
RBC (10^{12}/L)	7.79±0.42	7.96±0.62
HGB (g/L)	142.17±9.83	144.00±14.01
HCT (%)	44.38±2.91	44.58±6.09
MCV (fL)	56.98±2.97	55.92±4.56
MCH (pg)	18.26±1.09	18.08±0.88
MCHC (g/L)	320.17±4.71	324.17±12.16
PLT (10^9/L)	638.00±86.67	655.50±148.47
RDW-SD (fL)	38.38±1.24	40.60±4.48
RDW-CV (%)	21.61±0.97	22.97±2.26
NEUT# (10^9/L)	4.65±1.23	6.17±3.13
LYMPH# (10^9/L)	13.37±2.30	15.81±6.89
MONO# (10^9/L)	1.34±0.24	1.23±0.37
EO# (10^9/L)	0.63±0.43	0.65±0.53
BASO# (10^9/L)	0.17±0.07	0.21±0.06
NEUT%	23.15±5.39	25.80±11.72
LYMPH%	66.27±3.55	65.58±10.63
MONO%	6.70±0.70	5.32±1.24*
EO%	3.03±2.03	2.40±1.00
BASO%	0.85±0.40	0.90±0.19

Note: Compared with the normal, *$P < 0.05$, **$P < 0.01$.

Weight, FPG, 2hPG and glycometabolism related indicators

The levels of FPG, 2hPG, HbA1c and HOMA-IR in hyperglycemic BMPs were significantly higher than those of control subjects ($p < 0.05$); however, there were no significant differences in body weight, INS and GC between the two groups (Table 1).

Blood physiological indexes

There were no significant differences in blood physiological indexes between the two groups (Table 2).

Blood biochemical parameters

CHOL and LDL-CH levels in the hyperglycemia group were significantly higher than those of the NC ($P < 0.01$). However, there were no significant differences in other blood biochemical indexes between the two groups (Table 3).

DISCUSSION

Previously reported DM susceptible swine models include Yucatan Miniature Swine and Ossabaw Island Hog with low blood sugar clearance rate, and Iberian pigs with inheritable elevated levels of low density lipoprotein cholesterol (IHLC) and leptin resistance compared with Iberian pigs (Spurlock & Gabler 2008; Yuan & Tao 2016). The mutation of pig IHLC originated from a missense mutation (C253 → T253) in the LDL receptor, resulting in the substitution of Arg94 by Cys94 in the LDL. Moreover, the mutation was shown to be inherited in an autosomal manner, similar to that of human familial hypercholesterolemia. Spontaneous dyslipidemia is also characteristic of insulin resistant individuals, and has the potential to model human type 2 DM (Chen *et al.* 2009).

Most of the miniature pigs in China, especially Bama minipigs, are bred for food supply. Therefore, BMPs can tolerate most causative factors of diabetes such as lack of exercise and excess energy with rare natural occurrence (Yuan & Tao 2016). ENU, a synthetic chemical, is able to generate random and single base mutations in DNA sequences. The mechanism is that the acetyl group of ENU could replace chemical groups on some bases of the DNA. The male BMP 2907 was generated from crossing an ENU-induced male BMP with a normal female BMP, and hyperglycemic individuals were found from 2907 and its offspring. We assume that the hyperglycemic characteristics of the family are likely induced by ENU mutagenesis, which is similar to that of IHLC pigs. At present, considering the inheritability of hyperglycemia in G3, G4 and G5 generations, FPG levels in G4 and G5 generations were significantly higher than those of G3, and the G5 generation had glucose-tolerant individuals just like 2907 (G1). Therefore, it is presumed that in subsequent breeding generations, new families of diabetic miniature pigs would be established.

Compared with normal BMPs under the same environmental, feeding and age conditions, the G5 of the DM susceptible family showed higher levels of FPG, 2hPG and HbA1c. *In vivo*, blood glucose levels are mainly regulated by neurohumoral mechanisms, with insulin and glucagon as the main players. For DM, type 1 DM is mainly due to islet beta cell damage resulting in insufficient insulin secretion. In contrast, Type 2 DM mostly results from insulin resistance and islet beta cell dysfunction (Bellinger *et al.* 2006). Studies have shown that type 2 DM not only shows abnormal islet beta cells, but also abnormal islet alpha cells; abnormal glucagon secretion also promotes the occurrence of hyperglycemia and diabetes (Matthews *et al.* 1985). In this study, hyperglycemia group BMPs showed higher blood glucose levels, but normal insulin and glucagon levels, indicating that in this case hyperglycemia is not related to abnormal insulin or glucagon. The HOMA-IR values of the hyperglycemia group were significantly higher than those of the controls, indicating that BMPs in the hyperglycemia group had obvious insulin resistance, resulting in reduced response and sensitivity of tissues and cells to insulin, leading to decreased insulin-mediated glucose uptake and utilization efficiency, thus maintaining a high blood sugar phenotype (Liu *et al.* 2007). There are many factors and mechanisms underlying insulin resistance, including obesity, oxidative

Tab. 3. Blood biochemical indexes

	Normal (n=6)	Hyperglycemia (n=6)
TP (g/L)	72.91±4.76	75.23±6.00
ALB (g/L)	47.90±3.65	46.87±2.79
GLO (g/L)	25.02±7.59	28.37±3.91
A/G ratio	2.05±0.58	1.68±0.19
IBIL (μmol/L)	0.32±0.24	0.35±0.23
DBIL (μmol/L)	0.47±0.25	0.62±0.33
TBIL (μmol/L)	0.78±0.26	0.97±0.42
CHOL (mmol/L)	1.50±0.31	2.12±0.44**
LDL-CH (mmol/L)	0.80±0.27	1.23±0.34**
HDL-CH (mmol/L)	0.81±0.08	0.95±0.19
TG (mmol/L)	0.38±0.09	0.47±0.11
AST (U/L)	50.00±17.09	39.83±13.41
ALT (U/L)	64.50±8.14	61.00±17.56
AST/ALT ratio	0.78±0.28	0.72±0.42
CK (U/L)	738.50±409.09	936.83±888.41
LDH (U/L)	631.17±83.00	693.17±174.51

Note: *$P < 0.05$ and **$P < 0.01$, compared to the normal group.

stress, receptor substrate phosphorylation and organelle dysfunction (Koopmans *et al.* 2006). Further study is required to unveil the mechanism of insulin resistance in 2907 family.

Glucose tolerance is an indicator of the body's ability to regulate blood sugar. Once glucose tolerance is altered, the odds of developing DM increases. Oral glucose tolerance test (OGTT) is usually used to evaluate glucose tolerance in clinical practice. However, it is inconvenient and error-prone in terms of manipulating experimental minipigs. Therefore, IVGTT was used to evaluate glucose tolerance in minipigs. The results showed that pigs in the G5 generation showed impaired glucose tolerance, whereas other pigs had normal regulation of blood glucose.

The occurrence of diabetes is often accompanied by changes in physiological and biochemical indicators. Alken Tacitimur *et al.* found that the percentage of monocytes, hemoglobin, mean red blood cell volume and average platelet distribution width in patients with diabetes are significantly lower than those of normal individuals (Tobisch *et al.* 2011); Fang Li *et al.* found that platelet count (PLT) in DM patients is lower than that of healthy individuals, while platelet distribution width (PDW), mean platelet volume (MPV) and platelet ratio (P-LCR) are higher than those of healthy people (Li *et al.* 2010). Therefore, the occurrence of diabetes may possibly be monitored by detecting blood physiological indicators. In this study, there were no significant differences in blood physiological indexes between those two groups, probably due to the young age at testing that may mask abnormalities to develop in older age. The changes of biochemical indexes were reflected by abnormal metabolism of lipids, mainly including CHOL, TG, HDL-CH and LDL-CH. Several clinical studies have shown that diabetic patients have increased TG and LDL-CH, and decreased HDL-CH (Mooradian 2009); therefore, early prevention of diabetes can be achieved by monitoring blood lipids. The occurrence of dyslipidemia is related to insulin resistance, which affects TG and HDL-CH regulation (Zhang *et al.* 2016). In this study, TG and HDL-CH levels between those two groups were not significantly different; however, CHOL and LDL-CH levels in the hyperglycemia group were higher than those of the control group, consistent with blood lipid test results in DM patients, suggesting lipid metabolic disorders is indeed associated with DM.

The number of germ cells in male animals is much greater than that of female counterparts, which makes males more advantaged in ENU induction, resulting in more gametes mutated and larger amounts of subsequent mutated offspring (Aigner *et al.* 2008; Hai *et al.* 2017). It is critical to maintain and apply the established mutated family to carry out related studies. With the increasing age of 2907, we will focus to select suitable boars exhibiting similar or more severe hyperglycemic traits in 2907 derived generations. Most minipig breeds in China, including Bama minipigs, are found in a closed environment, which enables inbreeding to an extent (Yuan & Tao 2016). The 2907 family members were inbred to the 5th generation in good conditions without obvious defects from inbreeding. One of the G5 BMPs in the hyperglycemia group reached the test standard for DM, and more than 80% of BMPs at G5 were susceptible to DM, although the total number of family members remains small. Considering the general law of inbreeding, sterility and stillbirth would begin to occur in G5 or G6. The 2907 family could be utilized in DM modeling in two ways: (1) inbreeding should be continued to generate more stable DM susceptible families if no obvious defects are observed from inbreeding; (2) avoidance of further inbreeding of this line and induction with a high glucose/high fat diet or STZ to generate a DM model for scientific research.

In summary, the 2907 family has been initially established as DM susceptible. The selected hyperglycemia minipigs bred in this family show characteristics suggesting type 2 diabetes, including continuous increase in FPG, insulin resistance and lipid metabolism impairment. The family has the potential for further inbreeding selection or additional induction to establish DM model families.

DECLARATIONS

Ethical approval

The experiments were approved by the welfare and ethics committee and completed in general large

animal laboratory of Dongguan Songshan Lake Pearl Laboratory Animal Science and Technology Co., Ltd.

Conflict of interests

All authors declare that they have no any conflict of interest.

Fundings

Funds for PHD researchers of Guangdong Medical University in 2021 (GDMUB2021017). This study was supported by Major Scientific Problem-oriented Project under the National Key Basic Research Program (973 Program) (2011CBA01006), Science project of Guangdong province (2013B030300040 and 2013B060300001), Scientific research foundation of Guangdong medical university (2XB14003) and Key Program for International S&T Cooperation Projects of China (2011DFA33290).

Author contribution

guarantor of integrity of the entire study: Weiwang Gu
study concepts: Xiaohua Su
study design: Xiaohua Su
definition of intellectual content: Xiaohua Su
literature research: Juncheng Deng
clinical studies: Bayaer Nashun
experimental studies: Min Yue
data acquisition: Ming Lv
data analysis: Xiaohua Su
statistical analysis: Xiaohua Su
manuscript preparation: Xiaohua Su
manuscript editing: Xiaohua Su
manuscript review: Xiaohua Su

REFERENCES

1 Acharjee S, Ghosh B, Al-Dhubiab BE, Nair AB. (2013) Understanding type 1 diabetes: etiology and models, Can J Diabetes. **37**: 269–276.
2 Aigner B, Rathkolb B, Herbach N, Hrabé de Angelis M, Wanke R, Wolf E. (2008) Diabetes models by screen for hyperglycemia in phenotype-driven ENU mouse mutagenesis projects, Am. J. Physiol. Endocrinol. Metab. **294**: E232–240.
3 Bassols A, Costa C, Eckersall PD, Osada J, Sabrià J, Tibau J. (2014) The pig as an animal model for human pathologies: A proteomics perspective, Proteomics Clin Appl. **8**: 715–731.
4 Bellinger DA, Merricks EP, Nichols TC. (2006) Swine models of type 2 diabetes mellitus: insulin resistance, glucose tolerance, and cardiovascular complications, ILAR J. **47**: 243–258.
5 Chen H, Liu YQ, Li CH, Guo XM, Huang LJ. (2009) The susceptibility of three strains of Chinese minipigs to diet-induced type 2 diabetes mellitus, Lab animal. **38**: 355–363.
6 Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW6, Malanda B. (2018) IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045, Diabetes Res. Clin. Pract. **138**: 271–281.
7 Glovaci D, Fan W, Wong ND. (2019) Epidemiology of Diabetes Mellitus and Cardiovascular Disease, Curr Cardiol Rep. **21**: 21.
8 Hai T, Cao C, Shang H, Guo W, Mu Y, Yang S, Zhang Y, Zheng Qet al (2017) Pilot study of large-scale production of mutant pigs by ENU mutagenesis, Elife. **6**.
9 Koopmans SJ1, Mroz Z, Dekker R, Corbijn H, Ackermans M, Sauerwein H. (2006) Association of insulin resistance with hyperglycemia in streptozotocin-diabetic pigs: effects of metformin at isoenergetic feeding in a type 2-like diabetic pig model, Metab. Clin. Exp. **55**: 960–971.
10 Li F, Yuan L, and Xu D. (2010) Platelet parameters in patients with diabetic nephropathy **7**: 982.
11 Liu Y, Wang Z, Yin W, Li Q, Cai M, Zhang C, Xiao J, Hou H, Li H, Zu X. (2007) Severe insulin resistance and moderate glomerulosclerosis in a minipig model induced by high-fat/ high-sucrose/ high-cholesterol diet, Exp. Anim. **56**: 11–20.
12 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.
13 Mooradian AD. (2009) Dyslipidemia in type 2 diabetes mellitus, Nat Clin Pract Endocrinol Metab. **5**: 150–159.
14 Narayan KM, Gregg EW, Fagot-Campagna A, Engelgau MM, Vinicor F. (2000) Diabetes--a common, growing, serious, costly, and potentially preventable public health problem, Diabetes Res. Clin. Pract. **50 Suppl 2**: S77–84.
15 Perleberg C, Kind A, Schnieke A (2018) Genetically engineered pigs as models for human disease, Dis Model Mech. **11**.
16 Renner S, Blutke A, Dobenecker B, Dhom G, Müller TD, Finan B, Clemmensen C, Bernau M, Novak I, Rathkolb B, Senf S, Zöls S, Roth M, Götz A, Hofmann SM, Hrabë de Angelis M, Wanke R, Kienzle E, Scholz AM, DiMarchi R, Ritzmann M, Tschöp MH, Wolf E. (2018) Metabolic syndrome and extensive adipose tissue inflammation in morbidly obese Göttingen minipigs, Mol Metab. **16**: 180–190.
17 Schimenti J, Bucan M. (1998) Functional genomics in the mouse: phenotype-based mutagenesis screens, Genome Res.. **8**: 698–710.
18 Spurlock ME, Gabler NK (2008) The development of porcine models of obesity and the metabolic syndrome, J. Nutr.. **138**: 397–402.
19 Strauss A, Moskalenko V, Tiurbe C, Chodnevskaja I, Timm S, Wiegering VA, Germer CT, Ulrichs K. (2012) Goettingen Minipigs (GMP): Comparison of Two Different Models for Inducing Diabetes, Diabetol Metab Syndr. **4**: 7.
20 Tobisch B, Blatniczky L, Barkai L. (2011) Correlation between insulin resistance and puberty in children with increased cardiometabolic risk, Orv Hetil. **152**: 1068–1074.
21 Wolf E, Braun-Reichhart C, Streckel E, Renner S. (2014) Genetically engineered pig models for diabetes research, Transgenic Res.. **23**: 27–38.
22 Wu J, Yan LJ. (2015) Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity, Diabetes Metab Syndr Obes. **8**: 181–188.
23 Yan X, Wu Y, Zhong F, Jiang Q, Zhou T, Guo Y, Yang X, Liang J, Joshua Liao D, Lan G. (2018) iTRAQ and PRM-based quantitative proteomics in T2DM-susceptible and -tolerant models of Bama mini-pig, Gene. **675**: 119–127.
24 Yuan J, and Tao H. (2016) The research progress of miniature pig diabetes mellitus model. **10**: 84.
25 Zhang Z, Turer E, Li X, Zhan X, Choi M, Tang M, Press A, Smith SR, Divoux A, Moresco EM, Beutler B. (2016) Insulin resistance and diabetes caused by genetic or diet-induced KBTBD2 deficiency in mice, Proc. Natl. Acad. Sci. U.S.A.. 113E6418-6418E6426.