

09/11/2020 Nagaya M., et al.

Supplemental information

Title: Genetically engineered pigs manifesting pancreatic agenesis with severe diabetes

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Number of supplement tables. 7 tables.

Supplemental Table 1. Characteristics of the target genes evaluated using the real-time polymerase chain.

Gene symbol	Primer/ Probe	Sequences (5'-3')	Amplicon size (bp)	Ref Seq ID
PDX1	Forward	5'-GTGGATGAAGTCTACCAAGGCTC	72	NM_001141984
	Reverse	5'-TCGGGCTCTGCTGCGTAG		
	Probe	5'-CACCGCCTGCCCACTGGCCTTTC		
INS	Forward	5'-GCTTCTTCTACACGCCCAAGG	140	NM_001109772 AF064555
	Reverse	5'-GCACTGCTCCACGATGCC		
	Probe	5'-CGGAGAACCCTCAGGCAGGTGCC		
GCG	Forward	5'-CCAGTGACTACAGCAAGTATCTG	130	NM_214324
	Reverse	5'-GTCCCTTCAGCATGTCTCTCA		
	Probe	5'-CTTGTTCCCTCTTGGTGTTTCATCAGCCACTG		
SST	Forward	5'-CGCTGGGAAGCAGGAACTG	134	NM_001009583
	Reverse	5'-GCAGCTCCAGCCTCATTTCA		
	Probe	5'-CAAATCTTCAGGCTCCAGGGCATCGTTCT C		
ACTB	Forward	5'-TGGATGACGATATTGCTGCGC	132	XM_003124280
	Reverse	5'-CCATCACGCCCTGGTGTC		
	Probe	5'-AGCATCGTCGCCCGCAAAGCCG		

PDX1, pancreatic duodenum homeobox 1; INS, insulin; GCG, glucagon; SST, somatostatin; PPY,

09/11/2020 Nagaya M., et al.

pancreatic polypeptide; ACTB, β -actin; PCR, polymerase chain reaction; Ref SeqID, Reference Sequence Identification.

Supplemental Table 2. Whole pancreas and body weights at specified times.

Age	Pancreas weight (PW)			Body weight (BW)			PW/BW		
	Tg (g)	WT (g)	<i>P</i> value	Tg (g)	WT (g)	<i>P</i> value	Tg	WT	<i>P</i> value
Day1	0.2 ±	1.9 ±	0.0005 ^{***}	880.5±	1423.0 ±	0.0003 ^{***}	0.0%	0.1%	0.0003 ^{***}
	0.1	0.3 [*]		24.3	72.6		(0.03%)	(0.13%)	
Week 9	4.9 ±	53.9 ±	0.0009 ^{***}	20000.0	33625.0	0.01 ^{**}	0.0%	0.2%	0.0001 ^{***}
	0.6	10.3		± 2582.4	± 2656.6		(0.02%)	(0.16%)	
Week 16	18.7 ±	62.6	-	26650.0	62600	-	0.1%	0.10%	-
	1.8			± 2550.0			(0.07%)	(0.10%)	

The time after birth is indicated as days or weeks.

PW/BW ratio in Tg versus WT pigs.

Tg, transgenic pigs; WT, wild-type pigs.

Tg, 1 day, 9 weeks, and 16 weeks: n = 4, 3, and 2, respectively.

WT, 1 day, 9 weeks, and 16 weeks: n = 3, 4, and 1, respectively.

Quantitative data are presented as means ± SDs. ^{**}*P* < 0.01, ^{***}*P* < 0.001 versus WT.

Supplemental Table 3. Body and whole pancreas weights for Tg pigs with CSII.

Age	pancreas weight (PW)		Body weight (BW)		PW/BW	
	Tg (g)	WT (g)	Tg (g)	WT (g)	Tg	WT
36 weeks	48	153.3 ± 40.3	120000	131433.3 ± 14609.7	0.0% (0.04%)	0.1% (0.12%)

The time after birth is indicated as days or weeks.

PW/BW ratio in Tg versus WT pigs.

Tg, transgenic pigs; WT, wild-type pigs.

Tg: n=1.

WT: n=3.

Quantitative data are presented as means \pm SDs.

Supplemental Table 4. Biochemical parameters of blood samples.

	Day1			Week 4			Week 8		
	Tg	WT	P value	Tg	WT	P value	Tg	WT	P value
TP (g/dl)	5.9 \pm 0.5	5.8 \pm 0.8	0.92	4.5 \pm 0.5	4.7 \pm 0.2	0.46	5 \pm 0.5	5.2 \pm 0.5	0.66
ALB (g/dl)	1.6 \pm 0.1	1.5 \pm 0.2	0.83	3.2 \pm 0.6	2.9 \pm 0.3	0.42	3.9 \pm 0.7	4.3 \pm 0.6	0.39
TBIL (mg/dl)	0.7 \pm 0.2	0.2 \pm 0.0	0.03*	0.7 \pm 0.5	0.4 \pm 0.0	0.06	0.2 \pm 0.1	0.2 \pm 0.1	0.69
AST (IU/l)	38.7 \pm 3.4	49.7 \pm 18.1	0.45	24 \pm 6.9	44 \pm 8	0.002	27 \pm 13.4	28.9 \pm 13.4	0.82
ALT (IU/l)	45 \pm 13.4	29.7 \pm 4.6	0.2	45.4 \pm 11.3	59.3 \pm 6.5	0.07	36.1 \pm 3.5	37.3 \pm 5.2	0.66
ALP (IU/l)	6820.7 \pm 609.6	4475.7 \pm 152.7	0.01*	2316.6 \pm 486.5	1345 \pm 72.9	0.000983 38***	1036.6 \pm 427.6	1017 \pm 175.5	0.92
γ -GTP	64 \pm 9.9	50.3 \pm 4.6	0.15	41.4 \pm	27.5 \pm 1.1	0.01	38.3 \pm 10	26.8 \pm 4.0	0.03

09/11/2020 Nagaya M., et al.

(IU/l)				10.8					
LDH	697± 170	506.3± 85	0.23	520.1± 172.9	601± 138.7	0.47	526± 39.5	746.5± 103	0.0036
(IU/l)									
TCHO	96± 20.1	75.7± 7.8	0.25	77.3± 12.4	87± 21.7	0.39	89.3± 11.2	76.5± 20.3	0.21
(mg/dl)									
TG	38± 24.2	59.3± 6.3	0.29	202.6± 170.5	69± 9.8	0.08	132.7± 149.3	29.3± 15.9	0.14
(mg/dl)									
HDL-C	49.7± 10.3	28.3± 2.9	0.048	48.5± 10.4	44.5± 15.1	0.64	48.7± 8.5	37.2± 9.5	0.057
(mg/dl)									
GLU	816.3± 45.0	109.7± 11.3	0.00003*	438± 113.7	102.5± 6.4	0.000100	370.1± 255.7	109.3± 26.2	0.047*
(mg/dl)			**			99***			
Insulin									
(µIU/ml)	N.D.	2.8± 3.1	-	N.D.	0.9± 0.2	-	N.D.	5.3± 1.3	-
)									
AMYL	1466.3± 468.7	1035± 203	0.3	1273.1± 787.8	1302.3± 294.5	0.95	1294.7± 773.9	1582.8± 402.0	0.47
(IU/l)									
LIP	47.7± 3.9	43.7± 5.2	0.43	37.8± 5.3	45.3± 15.2	0.28	37.9± 7.8	35.0± 13.6	0.67
(IU/l)									
BUN	62.6± 12.2	18.5± 4.8	0.01*	18.3± 7.6	15.4± 2.5	0.39	12.7± 5.0	9.3± 2.0	0.17
(mg/dl)									
CRE	0.9± 0.0	0.6± 0.0	0.01	0.4± 0.0	0.6± 0.1	0.1	0.9± 0.2	0.7± 0.1	0.11
(mg/dl)									
UA	2± 0.2	0.4± 0.0	0.001	0.3± 0.1	0.4± 0.1	0.46	0.3± 0.1	0.3± 0.1	0.89
(mg/dl)									

6

09/11/2020 Nagaya M., et al.

Na (mEq/l)	144± 3.6	142.7± 2.9	0.7	129.1± 4.7	139.5± 0.9	0.000472 092	135.6± 3.7	139.2± 6.6	0.28
K (mEq/l)	3.9± 0.1	3.6± 0.3	0.33	4.4± 0.3	4.0± 0.2	0.08	4.4± 0.6	3.6± 0.2	0.02
Cl (mEq/l)	105.3± 3.3	100± 2.4	0.14	95.5± 4.3	98.0± 1.2	0.32	99.6± 4.0	101.3± 6.0	0.57
Ca (mg/dl)	12.2± 0.5	12.1± 0.0	0.86	9.3± 0.5	10.1± 0.5	0.03	10.1± 0.4	9.8± 0.8	0.41
Mg (mg/dl)	2.8± 0.2	2.1± 0.0	0.01	2.1± 0.1	2.4± 0.1	0.01	2.4± 0.2	3.6± 2.8	0.37
IP (mg/dl)	11± 0.3	8.3± 1.1	0.03	9.6± 0.7	10.5± 0.3	0.04	9.8± 0.3	9.7± 0.9	0.81

	Week 9			Week 16		
	Tg	WT	P value	Tg	WT	P value
TP (g/dl)	5.5± 0.2	5.4± 0.2	0.64	5.2± 0.2	4.9± 0.3	0.39
ALB (g/dl)	4.3± 0.2	4.2± 0.1	0.48	3.8± 0.2	2.9± 0.3	0.07
TBIL (mg/dl)	0.7± 0.2	0.1± 0.0	0.00158***	0.6± 0.4	0.2± 0.1	0.26
AST (IU/l)	57.0± 24.5	37.8± 6.6	0.26	23.7± 5.8	39.0± 3.0	0.08
ALT (IU/l)	44.7± 1.7	41.8± 2.8	0.23	36.0± 5.0	37.0± 5.0	0.88

09/11/2020 Nagaya M., et al.

ALP (IU/l)	1420± 201.7	962.8± 75.7	0.02*	888.0± 158.2	920.0± 21.0	0.84
γ-GTP (IU/l)	50.0± 5.7	38.3± 6.4	0.09	34.7± 4.2	34.5± 3.5	0.97
LDH (IU/l)	546.7± 61.8	783.5± 87.8	0.02	472.3± 161.4	739.0± 44.0	0.18
TCHO (mg/dl)	73.0± 5.1	62.8± 15.9	0.41	104.7± 4.1	67.5± 7.5	0.01
TG (mg/dl)	273.0± 94.4	61.5± 36.2	0.02	73.3± 30.4	30.0± 3.0	0.22
HDLC (mg/dl)	34.3± 7.0	33.3± 5.7	0.86	47.7± 3.3	33.0± 8.0	0.12
GLU (mg/dl)	517.0± 49.6	125.3± 16.3	0.00006***	382.0± 56.3	105.5± 5.5	0.01*
Insulin (μIU/ml)	N.D.	4.9± 1.1	-	N.D.	5.2± 1.0	-
AMYL (IU/l)	922.0± 348.8	1609.8± 593.7	0.19	1491.3± 658.7	1114.0± 110.0	0.58
LIP (IU/l)	64.3± 34.9	28.3± 7.1	0.28	27.7± 3.7	28.5± 6.5	0.9
BUN (mg/dl)	29.3± 2.5	6.6± 0.7	0.00003***	8.7± 4.0	9.2± 1.9	0.91
CRE (mg/dl)	1.2± 0.3	1.0± 0.1	0.27	1.1± 0.1	1.2± 0.0	0.73
UA	0.2± 0.0	0.2± 0.0	0.15	0.2± 0.0	0.3± 0.1	0.79

(mg/dl)						
Na						
(mEq/l)	134± 0.0	143± 1.6	0.00041	134.0± 3.6	143.5± 0.5	0.06
K (mEq/l)	4.6± 0.0	3.8± 0.3	0.01	5.1± 0.7	4.2± 0.4	0.27
Cl (mEq/l)	91.0± 1.4	103.5± 1.8	0.00039	98.7± 3.7	100.5± 0.5	0.63
Ca (mg/dl)	9.7± 0.9	10± 0.5	0.62	8.8± 0.9	9.1± 0.7	0.75
Mg						
(mg/dl)	3.1± 0.1	2.4± 0.2	0.01	2.5± 0.8	2.5± 0.0	1
IP (mg/dl)	9.6± 0.5	10± 1.0	0.64	10.1± 1.4	8.3± 0.7	0.3

The time after birth is indicated as days or weeks.

Tg, transgenic pigs; WT, wild-type pigs.

TP, total protein; ALB, albumin; TBIL, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ -GTP, gamma-glutamyl transferase; LDH, lactate dehydrogenase; TCHO, total cholesterol; TG, triglycerides; HDLC, high-density lipoprotein cholesterol; GLU, glucose; AMYL, amylase; LIP, lipase; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; Na, sodium; K, potassium; Cl, chloride; Ca, calcium; Mg, magnesium; IP, inorganic phosphate.

Tg, 1 day and 4 and 8 weeks: n = 3, 8, and 3, respectively.

WT, 1 day and 4 and 8 weeks: n = 3, 4, and 6, respectively.

Tg, 9 and 16 weeks: n = 3 and 4, respectively.

WT, 9 and 16 weeks: n = 4 and 2, respectively.

Quantitative data are presented as means \pm SDs. * P < 0.05, ** P < 0.01, *** P < 0.001 versus WT.

Supplemental Table 5. Biochemical parameters of blood samples from Tg pigs with CSII.

09/11/2020 Nagaya M., et al.

	Week 4		Week 8		Week 36	
	Tg	WT	Tg	WT	Tg	WT
TP (g/dl)	5.1	4.7± 0.2	5.5	5.2± 0.5	6.3	5.8± 0.8
ALB (g/dl)	3.3	2.9± 0.3	4.3	4.3± 0.6	4.3	3.8± 0.5
TBIL (mg/dl)	1.2	0.4± 0.0	0.3	0.2± 0.1	0.9	0.1± 0.0
AST (IU/l)	54	44± 8	22	28.9± 13.4	62	32.0± 4.0
GPT (IU/l)	52	59.3± 6.5	33	37.3± 5.2	73	43.0± 11.0
ALP (IU/l)	1866	1345± 72.9	1588	1017± 175.5	643	294.0± 14.0
γ-GTP (IU/l)	83	27.5± 1.1	31	26.8± 4.0	28	34.0± 0.0
LDH (IU/l)	703	601± 138.7	597	746.5± 103	864	316.5± 27.5
TCHO (mg/dl)	71	87± 21.7	94	76.5± 20.3	76	48.0± 3.0
TG (mg/dl)	209	69± 9.8	93	29.3± 15.9	90	16.5± 11.5
HDL-C (mg/dl)	38	44.5± 15.1	45	37.2± 9.5	18	26.5± 2.5
GLU (mg/dl)	331	102.5± 6.4	351	109.3± 26.2	589	113.0± 26.0
Insulin (μIU/ml)	N.D.	0.9± 0.2	N.D.	5.3± 1.3	N.D.	3.3± 2.5
AMYL (IU/l)	1665	1302.3± 294.5	2320	1582.8± 402.0	1577	1241.0± 453.0
LIP (IU/l)	83	45.3± 15.2	12	35.0± 13.6	31	35.0± 13.0
BUN (mg/dl)	51.4	15.4± 2.5	22.3	9.3± 2.0	29.7	8.2± 0.9
CRE (mg/dl)	0.5	0.6± 0.1	0.6	0.7± 0.1	1.6	1.2± 0.3
UA (mg/dl)	0.5	0.4± 0.1	0.3	0.3± 0.1	0.4	0.4± 0.1
Na (mEq/l)	135	139.5± 0.9	134	139.2± 6.6	136	118.0± 1.0
K (mEq/l)	3.4	4.0± 0.2	3.7	3.6± 0.2	5.6	3.5± 0.3
Cl (mEq/l)	95	98.0± 1.2	97	101.3± 6.0	97	80.0± 2.0

10

09/11/2020 Nagaya M., et al.

Ca (mg/dl)	9.1	10.1± 0.5	10.3	9.8± 0.8	9.1	8.4± 0.2
Mg (mg/dl)	2.4	2.4± 0.1	2.2	3.6± 2.8	2.2	1.7± 0.2
IP (mg/dl)	9.9	10.5± 0.3	10.4	9.7± 0.9	7	6.5± 0.8

The time after birth is indicated as days or weeks.

Tg, transgenic pigs; WT, wild-type pigs.

TP, total protein; ALB, albumin; TBIL, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ -GTP, gamma-glutamyl transferase; LDH, lactate dehydrogenase; TCHO, total cholesterol; TG, triglycerides; HDLC, high-density lipoprotein cholesterol; GLU, glucose; AMYL, amylase; LIP, lipase; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; Na, sodium; K, potassium; Cl, chloride; Ca, calcium; Mg, magnesium; IP, inorganic phosphate.

Tg, 4, 8, and 36 weeks: n=1.

WT, 4, 8, and 36 weeks: n=4, 6, and 2, respectively.

Quantitative data are presented as means \pm SDs.

Supplemental Table 6. Biochemical parameters of urine samples.

	Day 1			Week 4			Week 8		
	Tg	WT	P value	Tg	WT	P value	Tg	WT	P value
TP (g/dl)	0.4± 0.1	0.5± 0.1	0.21	1.2± 0.3	0.7± 0.5	0.14	1.2± 0.0	1.3± 0.0	0.35
Alb (g/dl)	0.1± 0.0	0.1± 0.0	1	0.1± 0.0	0.1± 0.0	–	0.1± 0.0	0.1± 0.0	0.15
GLU (mg/dl)	1976± 78.6	45.3± 11.1	0.001**	571.3± 530.4	24.0± 3.9	0.17	714.0± 968.1	21.0± 1.6	0.42
Amylase	90±	7.0± 4.2	0.12	31.8±	68.3±	0.16	36.7±	74.0±	0.24

(IU/l)	60.2			13.6	37.4		20.2	37.6	
CRE	13.2±	13.0±		20.8±	35.5±		13.5±	55.3±	
(mg/dl)	3.3	3.8	0.94	16.8	12.9	0.27	8.9	28.0	0.09

	Week 9			Week 16		
	Tg	WT	P value	Tg	WT	P value
TP (g/dl)	1.5± 0.1	1.1± 0.0	0.053	0.3± 0.1	1.2± 0.1	0.0496*
Alb (g/dl)	0.1± 0.0	0.1± 0.0	0.29	0.1± 0.0	0.1± 0.1	–
GLU (mg/dl)	1099.7± 449.3	7.3± 0.4	0.08	1822.8± 391.7	17.0± 5.0	0.004**
Amylase (IU/l)	21.7± 13.0	95.1± 82.6	0.25	46.8± 50.7	69.5± 12.5	0.64
CRE (mg/dl)	5.4± 1.5	47.1± 37.2	0.15	32.1± 5.8	28.3± 5.1	0.60

The time after birth is indicated as days or weeks.

Tg, transgenic pigs; WT, wild-type pigs.

TP, total protein; ALB, albumin; GLU, glucose; AMYL, amylase; CRE, creatinine.

Data are presented as means ± SDs.

Tg, 1 day and 4 and 8 weeks: n = 3, 4, and 3, respectively.

WT, 1 day and 4 and 8 weeks: n = 3, 4, and 4, respectively.

Tg, 9 and 16 weeks: n = 3 and 4, respectively.

WT, 9 and 16 weeks: n = 4 and 2, respectively.

Quantitative data are presented as means ± SDs. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus WT.

Supplemental Table 7. Biochemical parameters of urine samples from Tg pigs with CSII.

	Week 36	
	Tg	WT
TP (g/dl)	0.2	0.3± 0.1
ALB (g/dl)	0.1	0.1± 0.0
GLU (mg/dl)	1995	24.5± 3.5
AMY (IU/l)	24	43.0± 7.0
CRE (mg/dl)	99	31.8± 1.8

The time after birth is indicated as days or weeks.

Tg, transgenic pigs; WT, wild-type pigs.

CSII, Continuous subcutaneous insulin infusion.

TP, total protein; ALB, albumin; GLU, glucose; AMYL, amylase; CRE, creatinine.

Data are presented as means ± SEs.

Tg: n=1.

WT: n=2.

Quantitative data are presented as means ± SDs.