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Breeding Scheme and Selection of Animals for DiaComp Experiments V.2

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We use this protocol and it's working

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Abstract

Summary

It is extremely important to measure quantitative parameters (e.g. plasma glucose, urinary albumin, etc.) in a way that minimizes the effects of any adventitious variables, such as seasonal changes, dietary differences, any differences in the degrees of backcrossing, or unplanned differences in genotypes. This means that, whenever possible, *mice of the critical genotypes should be obtained from genotypically identical matings carried out at or close to the same time.* Archival data are not appropriate.

The breeding scheme to generate the experimental animals can vary in complexity, depending on the fertility of the two genders, whether the ***Testgene*** is to be studied in homozygotes or in heterozygotes, and whether the diabetes is induced genetically or by STZ. However, it is important to remember that, when looking for the effects of the Testgene genotype on diabetic complications, *the aim is to compare the* ***Testgene*** $^{mutant/mutant}$ *(or* ***Testgene*** $^{mutant/wt}$*) diabetic animals with their diabetic litter mates that are wildtype at the* ***Testgene*** *locus (****Testgene*** $^{wt/wt}$*).*

Reference

Wang, C-H, Li, F, Hiller, S, Kim, H-S, Maeda, N, Smithies, O, and Takahashi, N. A modest decrease in endothelial NOS in mice comparable to that associated with human NOS3 variants exacerbates diabetic nephropathy. Proc. Natl. Acad. Sci. USA 108(5): 2070-2075 (2011) PMID: 21245338 PMCID: PMC3033253.

Diabetic Complications:

Cardiovascular

Nephropathy

Neuropathy

Retinopathy

Uropathy

Troubleshooting

1 When testing the effects of homozygosity for a mutant (such as a knockout) ***Testgene*** on diabetes induced by the dominant ***Ins2***^{Akita} mutation, a conservative and usually trouble-free breeding scheme is:

Testgene $^{mutant/wt}$ & ***Ins2*** $^{wt/wt}$ female (Inbred1) x ***Testgene*** $^{mutant/wt}$ & ***Ins2*** $^{Akita/wt}$ male (Inbred2)

Males and females of six genotypes result -- the three ***Testgene*** genotypes, each with or without diabetes:

1.	*Testgene* $^{mutant/mutant}$	:	*Ins2* $^{Akitat/wt}$	(Inbred or F1)
2.	*Testgene* $^{mutant/wt}$	:	*Ins2* $^{Akita/wt}$	(Inbred or F1)
3.	*Testgene* $^{wt/wt}$	:	*Ins2* $^{Akita/wt}$	(Inbred or F1)
4.	*Testgene* $^{mutant/mutant}$	:	*Ins2* $^{wt/wt}$	(Inbred or F1)
5.	*Testgene* $^{mutant/wt}$	:	*Ins2* $^{wt/wt}$	(Inbred or F1)
6.	*Testgene* $^{wt/wt}$	:	*Ins2* $^{wt/wt}$	(Inbred or F1)

Note that both parents, although inbred, are *heterozygotes for the mutant form of the Testgene*, which are almost always healthier and have more offspring than homozygous mutants. The offspring can be F1 hybrids (genetically as uniform as inbreds but hardier), or can be kept inbred if this is preferred. Note also that this mating produces the essential genotypes (usually 1 & 3) as littermates. Studying all six genotypes is very informative, but not essential. However, if *Testgene* heterozygotes are included (1,2, & 3), the functional consequences of different levels of expression of the ***Testgene*** on diabetic complications can be determined. If wildtype and mutant *Testgene* mice are studied on both diabetic and non-diabetic backgrounds (1,3,4 & 6), additive, super-additive or sub-additive interactions between the *Testgene* and diabetes can be detected.

In the case of diabetes induced by the Akita mutation in heterozygous state, it has been the DiaComp experience (1) that a higher level of chronic hyperglycemia is seen in males, and (2) that the development of diabetic complications is age-dependent and largely male-limited. Thus, the investigator could cull females as soon as sex can be determined and wean only males, thereby reducing costs and saving colony space.

When testing the effects of transgenes, it is generally advisable to avoid homozygosity since undesirable artifacts (insertional mutagenesis leading to homozygous lethality or other major physiologic/metabolic anomalies) can result independent of the presence or absence of diabetes.

When homozygous transgenes are not needed, or when the homozygous knockout of a ***Testgene*** is lethal, a simpler breeding scheme can be used:

Testgene $^{mutant/wt}$ & ***Ins2*** $^{wt/wt}$ female (Inbred1) x ***Testgene*** $^{wt/wt}$ & ***Ins2*** $^{Akita/wt}$ male (Inbred2)

Males and females of four genotypes result:

7.	*Testgene* $^{mutant/wt}$	:	*Ins2* $^{Akita/wt}$	(Inbred or F1)
8.	*Testgene* $^{wt/wt}$	:	*Ins2* $^{Akita/wt}$	(Inbred or F1)
9.	*Testgene* $^{mutant/wt}$	:	*Ins2* $^{wt/wt}$	(Inbred or F1)
10.	*Testgene* $^{wt/wt}$	:	*Ins2* $^{wt/wt}$	(Inbred or F1)

We append recent results that Dr. Nobuyuki Takahashi has obtained in an experiment to determine the effects of *Nos3* genotype on diabetic nephropathy (Wang et al., 2011). He studied all six male genotypes resulting from *Nos3*$^{+/-}$ *Ins2* $^{wt/wt}$ female (129SvEv) x *Nos3*$^{+/-}$ *Ins2* $^{Akita/wt}$ male (C57BL/6) matings. The data obtained from the resulting F1 mice are internally very consistent, and the error bars are relatively small. Interestingly the phenotypes of all six genotypes are distinguishable and progressively more severe in going from 6 to 1.

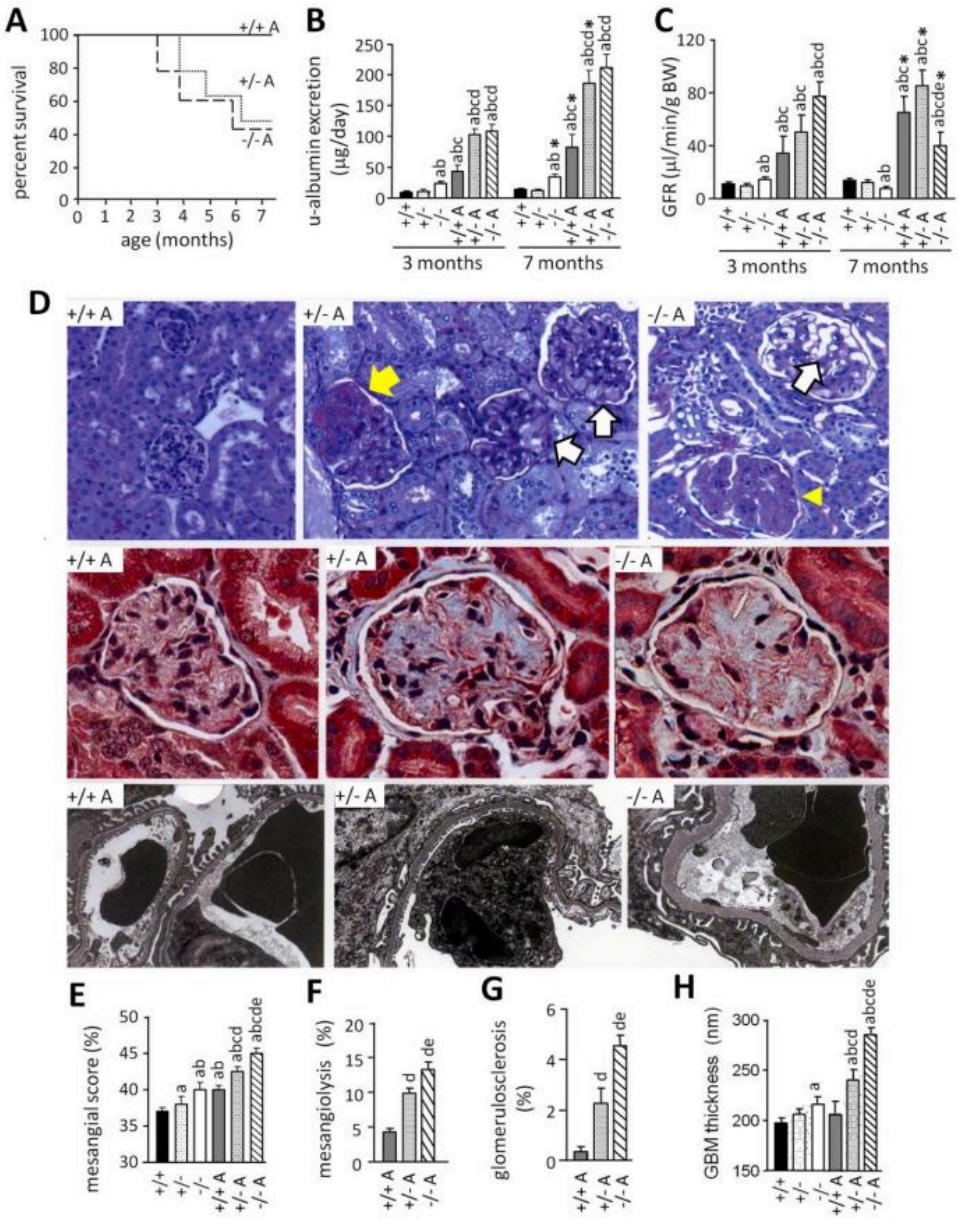

Figure 1. Survival rates, renal function, and histology of F1 (129SvEv x C57BL/6J) mice with different eNOS genotypes and Ins Akita mutation. A. Survival rates of three groups of diabetic mice with different eNOS genotypes. All non-diabetic mice regardless of the genotype of eNOS survived until the end of the experiment (not shown). B. Daily urinary albumin excretion when animals were at 3 months and 7 months old. C. GFR estimated by creatinine clearance when animals were at 3 months and 7 months old. D. Representative kidney morphology of 7 month-old diabetic mice with different eNOS genotypes. Top panels: PAS stain (original magnification x100). Yellow arrow: mesangial expansion; open arrow: mesangiolysis; yellow arrow head: glomerulosclerosis. Middle panels: glomeruli with Masson Trichrome stain (original magnification x200). Bottom panels: transmission electron micrographs with original magnification of x 12,500. E-H. Quantification of mesangial expansion (E), mesangiolysis (F), glomerulosclerosis (G), and GBM thickness (H) of 7 month-old mice. There was no glomerulosclerosis in any of non-diabetic mice regardless of eNOS genotypes. +/+, +/-, and -/- designate eNOS genotypes. A: Ins2Akita. Data are mean ± SEM. n≥8. a, b, c, d, e: $p<0.05$ vs. +/+, +/-, -/-, +/+ A, +/- A, respectively. *$p<0.05$ vs. values when the same mice were 3 months old.

Table 1 Characteristics of 3-month-old F1 (129SvEv x C57BL/6J) males having wild type and mutant *eNOS* and *Ins2* genes

Genotype	+/+	+/-	-/-	+/+ Akita	+/- Akita	-/- Akita	diabetes (D)	genotype (G)	DxG
BW (g)	29.4 ± 0.9	30.5 ± 0.8	28.0 ± 0.9	26.4 ± 0.9	28.0 ± 0.5	26.9 ± 0.7	0.002	N.S.	N.S.
BP(mmHg)	109.8 ± 2.3	127.6 ± 3.3^{a}	137.8 ± 4.0^{ab}	107.6 ± 3.6^{c}	126.8 ± 2.3^{acd}	142.8 ± 2.6^{abde}	N.S.	<0.0001	N.S.
glucose (mg/dl)	165 ± 19	199 ± 21	165 ± 20	422 ± 29^{abc}	467 ± 17^{abc}	515 ± 21^{abc}	<0.0001	N.S.	N.S.
food intake (g/day)	3.9 ± 0.5	4.6 ± 0.7	3.6 ± 0.7	5.1 ± 0.8	7.0 ± 0.5^{abcd}	8.4 ± 0.5^{abcd}	<0.0001	N.S.	0.05
water intake (ml/day)	3.0 ± 0.3	3.0 ± 0.2	3.0 ± 0.3	23.3 ± 3.6^{abc}	26.5 ± 2.0^{abc}	26.3 ± 2.6^{abc}	<0.0001	N.S.	N.S.
urine volume (ml/day)	1.5 ± 0.5	1.0 ± 0.2	1.3 ± 0.2	20.4 ± 1.6^{abc}	24.1 ± 2.5^{abcd}	21.1 ± 2.0^{abcd}	<0.0001	N.S.	N.S.

Data are mean ± SEM. n≥8. BW, body weight; BP: blood pressure; glucose: plasma glucose. GxD, designates *p* values of interaction between genotype and diabetes. N.S.: not significant. +/+, +/-, -/- designate eNOS genotypes. A: Akita. a,b,c,d,e: p<0.05 vs. +/+, +/-,-/-, +/+Akita, +/-Akita, respectively.

Table 2 Characteristics of 7-month-old F1 males having wild type and mutant *eNOS* and *Ins2* genes

Genotype	+/+	+/-	-/-	+/+ Akita	+/- Akita	-/- Akita	diabetes (D)	genotype (G)	DxG
BW (g)	33.2 ± 1.0	36.5 ± 1.3	37.8 ± 1.2	26.5 ± 1.2^{abc}	29.3 ± 1.2^{abcd}	33.3 ± 0.9^{cde}	0.002	<0.0001	N.S.
BP (mmHg)	117.0 ± 2.3*	127.0 ± 2.9^{a}	135.8 ± 2.9^{ab}	119.0 ± 2.9^{bc}*	134.0 ± 2.9^{abd}*	145.8 ± 2.7^{abcde}	<0.01	<0.0001	N.S.
glucose (mg/dl)	166 ± 16	203 ± 21	216 ± 20	465 ± 20^{abc}	500 ± 19^{abc}	514 ± 22^{abc}	<0.0001	N.S.	N.S.
food intake (g/day)	4.0 ± 0.4	3.8 ± 0.5	3.6 ± 0.5	9.5 ± 0.9^{abc}*	8.9 ± 0.8^{abc}*	10.8 ± 1.1^{abc}*	<0.0001	N.S.	<0.05
water intake (ml/day)	3.0 ± 0.2	2.7 ± 0.2	2.5 ± 0.2	25.5 ± 3.0^{abc}	31.9 ± 3.5^{abcd}	34.4 ± 2.7^{abcd}*	<0.0001	N.S.	N.S.
urine volume (ml/day)	1.0 ± 0.2	1.4 ± 0.2	1.2 ± 0.3	22.8 ± 3.0^{abc}	32.8 ± 3.5^{abcd}*	28.3 ± 2.5^{abcd}*	<0.0001	N.S.	N.S.
kidney weight (mg/g BW)	15 ± 2	14 ± 1	14 ± 1	19 ± 2^{abc}	20 ± 2^{abc}	21 ± 2^{abc}	<0.0001	N.S.	N.S.

Data are given as mean ± SEM. n≥8. BW, body weight. ; BP: blood pressure: glucose: plasma glucose. GxD, designates *p* values of interaction between genotype and diabetes. N.S.: not significant. +/+, +/-, -/- designate eNOS genotypes. A: Akita. a,b,c,d,e: p<0.05 vs. +/+, +/-, -/-,+/+Akita, +/-Akita, respectively. *p<0.05 vs. values when the same mice were 3 months old.