Animal Models of Diabetic Complications Consortium

Pilot Project

Annual Report
(2009)

"AN OUTBRED MOUSE STRAIN TO STUDY DIABETIC NEPHROPATHY"

Vanderbilt University School

Principal Investigator
Raymond C. Harris, M.D

To date, the investigators of the AMDCC have primarily characterized inbred laboratory mice strains to study the development diabetes nephropathy. These studies support the influence of genetic risk factors as contributing to diabetic nephropathy in these inbred lines. The potential disadavantage of using inbred lines is that the complexity of the genomes of inbred mouse strain are necessarily reduced, as compared to the genetic complexity of outbred human populations. Inbred mice are homozygous at all loci, whereas in outbred populations, loci allowed to exist in both heterozygous and homozygous states (1). Furthermore during the process of brother-sister mating used to derive inbred mouse strains, any polymorphisms that either decrease fecundity or are lethal when homozygous, are lost. If similar mutations contribute, in a dominant or semi-dominant fashion, to human diabetic nephropathy, this variability would never be seen in inbred mouse models. The goal of this pilot project was to begin to investigate the utility of outbred mice as potential models of diabetic nephropathy. Outbred mouse strains theoretically provide increased heterogeneity at loci that may predispose to development or progression of diabetic nephropathy. For these studies we examined development of diabetic nephropathy in the CD-1 strain, an outbred line that has been reported to be predisposed to development of diabetic nephropathy, and to initiate a backcross CD-1 mice with B6Akita mice, a strain resistant to development of diabetic nephropathy, in order to begin to identify potential dominant alleles that contribute to diabetic nephropathy. A previous study reported that CD-1 mice develop significant nephropathy in a high dose streptozotocin-induced model of diabetes (2), with prominent tubulointerstitial nephritis and fibrosis within 3 months and death because of diabetic complications by 6-7 months. The histopathologic lesions observed in these mice were reported to mimic human diabetic nephropathy, with glomerular hypertrophy, diffuse glomerulosclerosis, tubular atrophy, interstitial fibrosis and a progressive decrease in function (2). Our own preliminary data in CD-1 mice indicated significant diversity in the levels of albuminuria (following STZ induced diabetes utilizing the AMDCC protocol) among individual mice. Such heterogeneity suggested that outbred mice might more closely resemble the heterogeneity of human populations and might also provide the opportunity to capture dominant genes that contribute to increased albuminuria and diabetic nephropathy.

For these studies, we proposed two specific aims:

Specific Aim I: Characterization of the heterogeneity of diabetic nephropathy in CD-1 mice

Specific Aim II: Development of mouse lines exhibiting dominant inheritance of albuminuria and renal fibrosis

Specific Aim I: Characterization of the heterogeneity in diabetic nephropathy in CD-1 mice

Seventy-eight CD-1 mice (36 male and 41 female) were administered streptozotocin (STZ) (50mg/kg i.p. qd x 5d) at age 10 weeks. We established the presence of hyperglycemia by determining a six hour fasting blood sugar obtained at 2-4 weeks post STZ. Albuminuria and BUN were measured on spot urine ACR at the indicated times. FITC inulin measurements were obtained in selected mice at months 3, 6, 9 and 12 post STZ. After 8 weeks of diabetes, we had 36 male mice with blood sugars >200mg/dl (396±13) and 37 out of 41 females with blood sugars >200 mg/dl (318±14). Urine ACRs 8 weeks after onset of diabetes ranged from 44 to 851 µg alb/mg Cr in males and from 40 to 467 µg alb/mg Cr in females. There was no correlation with degree of hyperglycemia. These initial results were encouraging. In each sex, the 10 mice with the lowest ACRs and 10 with the highest ACRs were strikingly and significantly different. (male:low 68±4; high:389±65; female:low 64±2; high 365±30) **(Figure 1)**.

In general, the mice remained hyperglycemic during the time of study, although there was as trend for fasting blood sugars to decrease in both sexes (**Figure 2 and Table 1**)

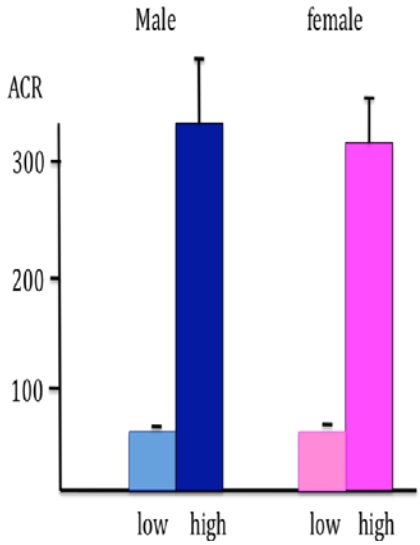

Figure 1 Mean ACRs at 8 weeks of the highest 10 and lowest 10 male and female diabetic CD-1 mice

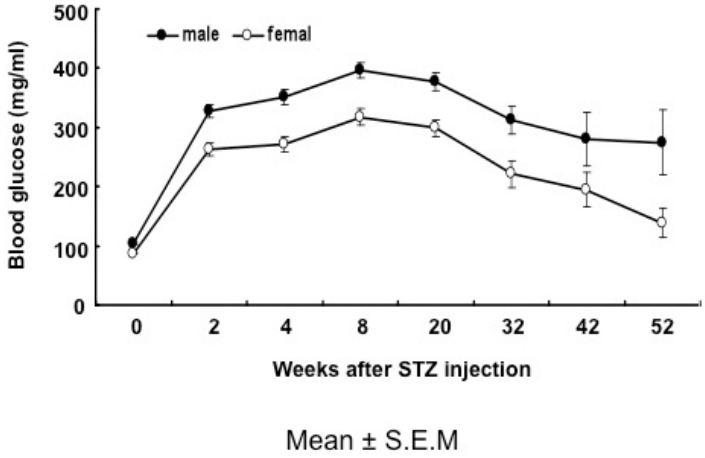

Figure 2 Fasting blood glucose in CD-1 mice made diabetic with the low dose STZ protocol

Table 1A

male

BG	0w	2w	4	8w	20w	31w	40w
X	104.0	328.1	351.3	396.1	377.0	312.4	280.8
sd	11.6	64.5	84.7	77.3	80.4	95.2	145.4
n	23	39	38	36	27	15	10
>200		37	36	36	26	12	

Table 1B

female

BG	0w	2w	4w	8w	20w	31w	40w
X	86.9	263.6	271.7	317.5	300.2	221.6	195.55
sd	9.6	68.7	83.1	89.3	82.2	120.0	134.2
number	23	41	41	41	38	28	20
BG>200		33	33	37	33	14	13

One of the most striking and unexpected findings was the high mortality rate (**Figure3**), with significantly greater mortality in the 10 mice with the highest ACR at 8 weeks than in the 10 mice with the lowest ACR at 8 weeks (**Figure 4**). The subsequent ACRs of the highest and lowest groups are shown in **Figure 5**.

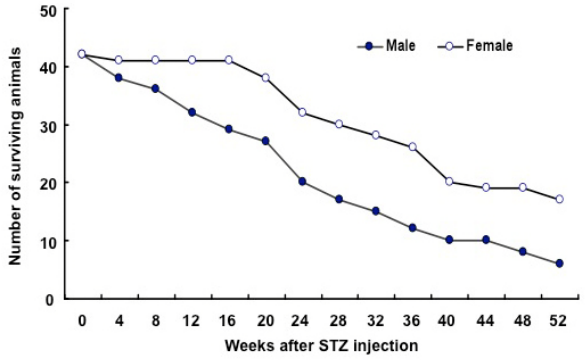

Figure 3 Survival Curve for all CD-1 diabetic mice

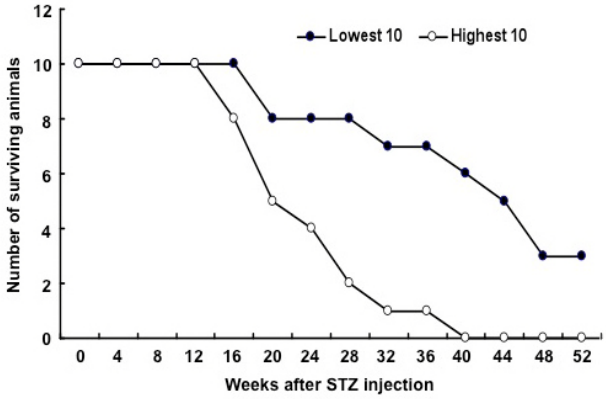

Figure 4 Survival Curve for the 10 CD-1 mice with the highest ACRs and the 10 with the lowest ACRs at 8 weeks of diabetes

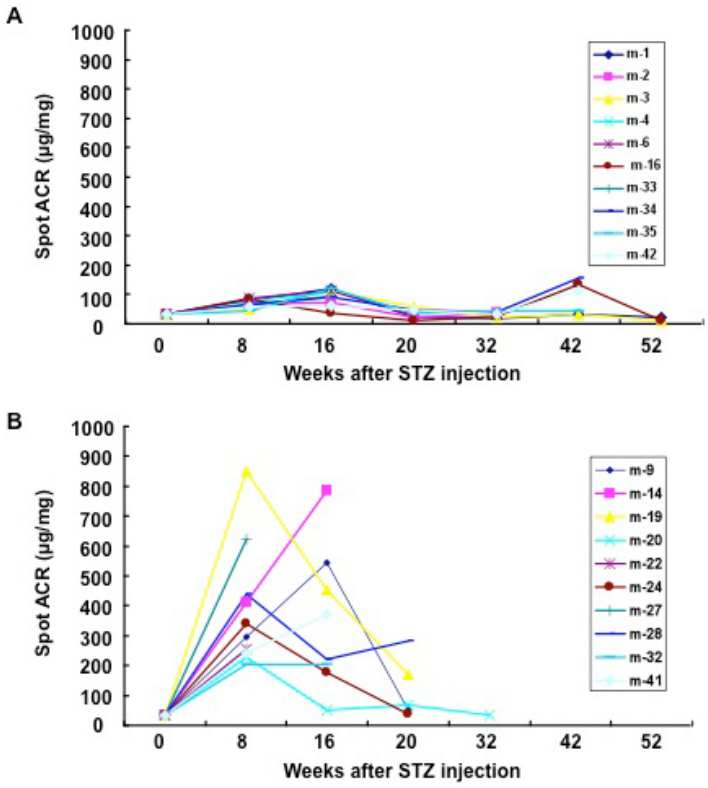

Figure 5 ACRs for the 10 CD-1 mice with the lowest ACRs (A) and the 10 with the highest ACRs (B) at 8 weeks of diabetes

We measured GFR by FITC inulin at 3 and 6 months. We will also measure at 12 months when we sacrifice the remaining mice. We did not see any significant trend in GFR in these mice at these earlier time points (**Figure 6**).

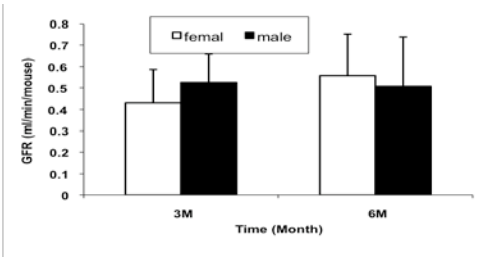

Figure 6 GFR of CD-1 mice 3 and 6 months after induction of diabetes with STZ

Similar results were obtained for the female CD-1 mice. We have just attained the 52 week mark and have measured GFRs and ACRs in the remaining mice and have sacrificed all mice for determination of renal histology. These results will be forthcoming in the next few weeks. We sacrificed a limited number of mice at 6 months. Preliminary histology indicates that there may be more mesangial expansion and tubulointerstitial injury in the mice with higher ACRs (**Figure 7**), but we will have to confirm this with more samples and by examination of the mice sacrificed at later time points.

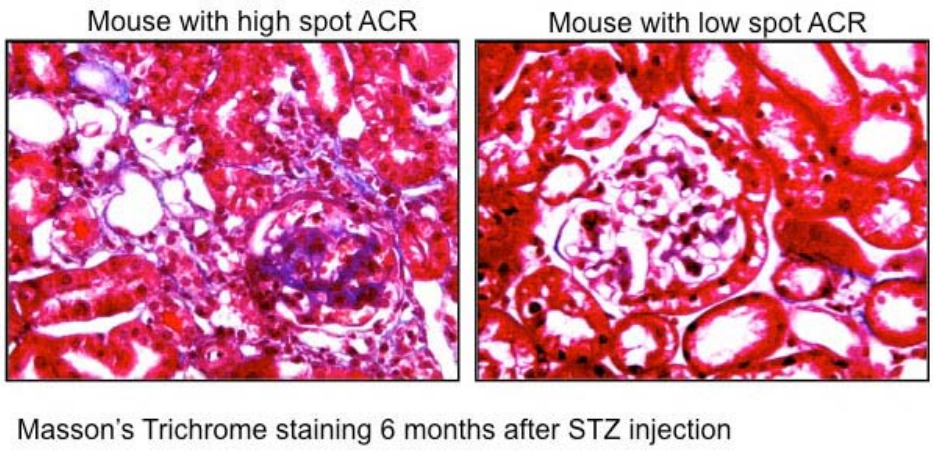

Figure 7 Masson-Trichrome staining of CD-1 diabetic mice sacrificed at 6 months of diabetes.

Specific Aim II*: *Development of mouse lines exhibiting dominant inheritance of albuminuria and renal fibrosis

Since outbred mice are bred to maintain heterozygosity at a maximal number of loci, dominant alleles that predispose to diabetic nephropathy mayexist in those mice developing robust albuminuria and renal fibrosis. Conversely these alleles should exist at reduced frequency in those mice with low ACR and fibrosis and protective alleles should be present. We and others have previously published that the C57BL6 inbred line is resistant to the development of nephropathy after induction of diabetes. We wished to take advantage of this resistance and the fact that the C57B6 genome has already been characterized to identify potential dominant risk alleles present in CD1 mice. To identify such dominant risk alleles, we have attempted to generate an F1 cross between the nephropathy resistant C57BL6 inbred line and the outbred CD-1 strain. These studies were performed by crossing female B6Ins2akita mice (a nephropathy resistant model of type I diabetes) with male CD-1 mice with the highest ACRs and males with the lowest ACR at 8 weeks (see above). This study was hampered by the high mortality of the males (see above) and their decreased mating behavior and/or fertility. However, we have obtained a number of offspring from these crosses that carry the Akita mutation and are diabetic, as evidenced by elevated blood sugars (see **Table 2**). To date, there is no consistent pattern of elevation in ACRs, but we will continue to follow these mice as well as continue to breed more of this F1 generation to determine if it will be worthwhile to consider further backcrosses and analysis.

Blood glucose and urine Alb/Cre ratio of F1 progeny that come from CD-1 male and Akita femal

				2m		5m	
	DOB	Father	F-A/C	BG	A/C	BG	A/C
AK4	3-9-09	m25	h	252	26.12	357	8.16
AK5	3-9-09	m25	h	307	53.08	363	11.85
AK6	3-15-09	m12	l	331	11.27	346	12.12
AK8	3-15-10	m12	l	333	9.80	317	8.49
AK13	3-11-09	m1	l	376	57.40	324	19.85
AK14	3-11-10	m1	l	329	15.58	292	32.67
AK16	3-11-11	m1	l	329	20.86	305	13.35
AK17	3-15-09	m16	l	314	10.28	dead	
AK18	**3-14-09**	**m39**	**h**	**312**	22.10	328	21.82
AK20	**3-14-09**	**m39**	**h**	**290**	12.41	330	45.16
AK21	4-4-09	m25	h	329	17.41	356	34.97
AK22	4-4-09	m25	h	246	8.68	303	76.23
AK26	4-6-09	m1	l	336	29.97	317	30.55
AK28	4-6-09	m1	l	274	23.21	275	40.18

References

1. Chia, R., Achilli, F., Festing, M.F., Fisher, E.M. The origins and uses of mouse outbred stocks. *Nat Genet* 37:1181-1186, 2005..

2. Sugimoto, H. Grahovac, G., Zeisber, M., Kalluri, R.Renal fibrosis and glomerulosclerosis in a new mouse model of diabetic nephropathy and its regression by bone morphogenic protein-7 and advanced glycation end product inhibitors. Diabetes 56:1825-33, 2007