Animal Model of Diabetic Complication Consortium
(GRANT NUMBER)

Annual Report
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"Effect of Diabetes on Prostate Inflammation and Epithelial Hyperplasia"

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Accomplishments

In 2010, our lab reported no significant difference of histological phenotype in DBA2J.Ins2-Akita mice contrast with control mice. A progression of inflammation was scored with samples of H&E staining of three different area; cranial, middle and caudal, by inflammatory grading standard with three criteria; inflammatory infiltrate, hyperplasia and vascular pathology. With that grading system, no significant difference was found in DBA2J.Ins2-Akita mice contrast with control mice. All of previous studies were reported by adding up all values together from three different lobes; CG, DLP and VP. To see the effects of diabetes on each prostatic lobe, three random areas per lobe per mouse were analyzed in 2011 by inflammatory grading system; inflammatory infiltrate, hyperplasia (shown in Table 1, same as progress report in 2010) and tissue damage (shown in Table 2, new criteria).

Intensity		**Inflammatory Infiltrate**	**Hyperplasia**	**Vascular Pathology**
0	Absent	No infiltrating leukocytes	Pseudostratified bilayer	No extravascular erythrocytes
1	Mild	< 10 leukocytes / field	Two clear layers-basal or luminal	Hemorrhage < 10 erythrocytes
2	Moderate	10-30 leukocytes / field	Expansion 3-5 epithelial cell layers	Hemorrhage 10-30 extravascular erythrocytes
3	Severe	> 30 leukocytes / field	Expansion greater than 5 epithelial cell layers	Hemorrhage > 30 extravascular erythrocytes

Focality		
0	Absent	No evidence of inflammatory criterion
1	Focal	Found in 1 location
2	Limited	Found in 1 – 25% of stromal tissue
3	Intermediate	Found in 25-50% of stromal or epithelial tissue
4	Widespread	Found in >50% of stromal/epithelial tissue; glandular infiltrate present

Table 1. Inflammatory grading chart.

Intensity		Tissue Damage
0	Absent	None
1	Mild	Sloughed epithelium (< 25% of tissue) and/or presence of pyknotic nuclei (1-20 per field)
2	Moderate	Sloughed epithelium (> 25% of tissue) and/or presence of pyknotic nuclei (>20 per field)
3	Severe	Loss of ductal epithelium

Focality		
0	Absent	No evidence of inflammatory criterion
1	Focal	Found in 1 location
2	Limited	Found in 1 – 25% of stromal tissue
3	Intermediate	Found in 25-50% of stromal or epithelial tissue
4	Widespread	Found in >50% of stromal/epithelial tissue; glandular infiltrate present

Table 2. Tissue damage grading chart.

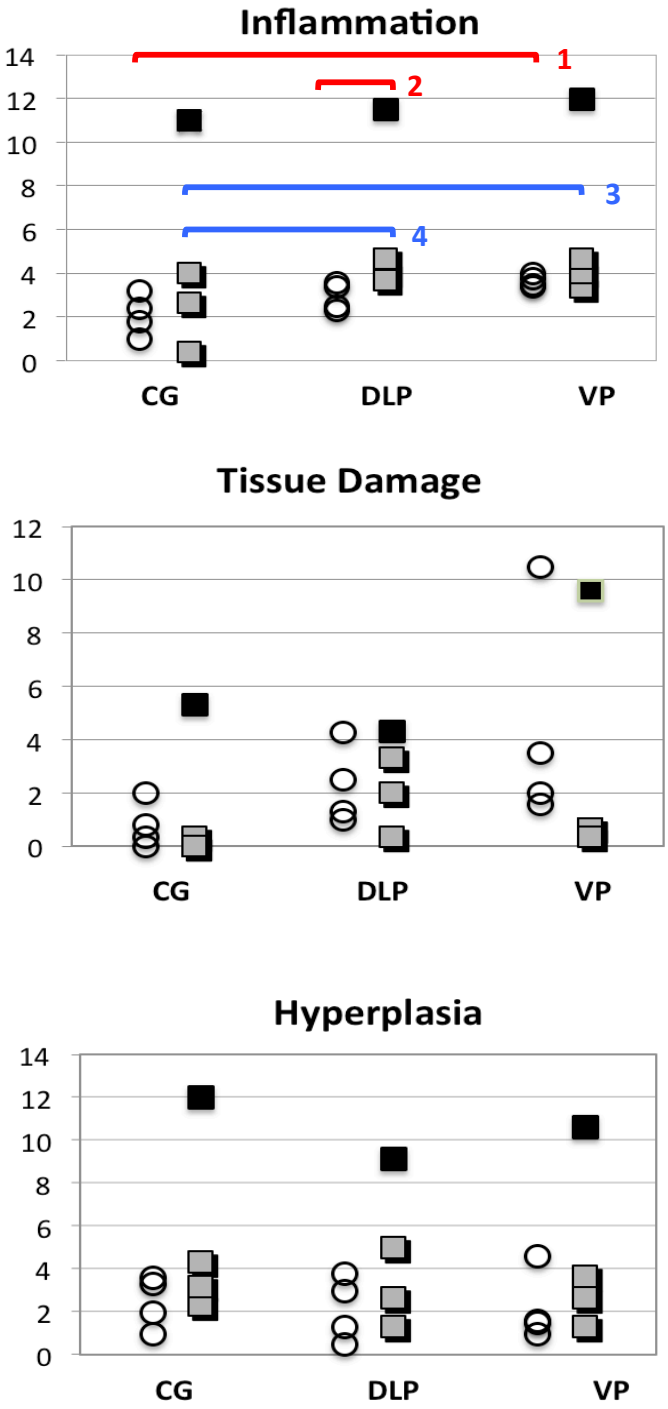

Figure 1. Comparison of inflammation (inflammatory infiltrate), tissue damage and hyperplasia between 18 weeks old wild type (n=4) and diabetic (n=4) mice in each prostatic lobe; coagulating gland(CG), dorsal lateral prostate (DLP) and ventral prostate (VP). Open circle: wild type, gray square: diabetic, black square: diabetic with severe inflammation. P-values: 1=0.029; 2=0.016; 3=0.040; 4=0.030. (Red line: comparison includes all animals in each group, Blue line: comparison excludes one diabetic with severe inflammation)

We employed analysis of variance (ANOVA) with multiple comparisons using Fisher's protected least significant difference tests. Prior to analysis, all values were rank-transformed in order to better meet the assumptions of ANOVA. P-values less than 0.05 were considered as significant. All analysis were performed using SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC). As shown in progress report in 2010, one diabetic showed severe inflammation in prostate and we postulated that it could be happened as a coincidence with environmental effects, possibly infection by exogenous pathogens, which are not direct effects due to diabetic complication. Here we provide dot charts which enable each score to be distinguished from others. Open circles indicate wild type, age matched control group. Gray squares indicate diabetic group with mild inflammation. Black square indicates one diabetic mouse showed severe inflammation.

With criteria of inflammatory infiltrate, DLP in diabetic mice showed significantly higher scores (P-value: 0.016) than aged matched wild type control (indicated as #2 in Figure 1). Among diabetic group without one diabetic mouse with severe inflammation, interestingly scores of DLP (p-value: 0.030, #4 in Figure 1) group and VP (p-value: 0.040, #3 in Figure 1) group were significantly higher contrast to CG. These results might suggest different level of inflammatory response in lobe specific manner in 18 weeks old adult mouse prostate with diabetic complication. With criteria of tissue damage and hyperplasia, no p-value for comparisons among each prostatic lobe in diabetic and age-matched control group was significant. Still, with both criteria, scores from one diabetic mouse with severe inflammation were highest compared to other diabetic mice.

Evidence for extracellular matrix (ECM) accumulation and basement membrane thickening with diabetic complication has been published. ECM is composed of collagens, elastins, structural glycoproteins, proteoglycans-hyaluronans and integrins. Collagens are the main components of ECM and defective collagens prevents the formation of strong connective tissue resulted as bleeding gums, loss of teeth, skin discoloration and wounds. With combination of Picrosirius Red Staining and polarized microscope, we observed collagens distribution and developed quantification analysis in 18 weeks old diabetic and age-matched control mice. Percentage of Picrosirius Red Staining positive area from Whole stack of images was quantified by WCIF Image J software. Interestingly, Picrosirius Red Staining positive area (%) of VP in diabetic mice was significantly higher than control mice (p-value: 0.008, indicated as #3 in Figure2). Without one diabetic mouse with severe inflammation, area (%) of VP in diabetic group was significantly higher compared to CG in diabetic group (#4 in Figure 2). Within the comparisons among each prostatic lobe in 18 weeks old wild type mice, DLP showed more collagen content compared to CG (p-value: 0.008, #1 in Figure 2) and VP showed more collagen content compared to DLP (p-value: 0.045, #2 in Figure 2).

In summary, our results suggest that type I diabetic complication followed by Akita gene modification impacts on inflammatory infiltrate in DLP and dramatic changes of collagen content in VP. Also, with dot chart presentation, each value from different prostatic lobe in diabetic vs wild type group can be shown which enables to notify the value from one diabetic mouse with severe inflammation and make comparisons in multiple different fashion. As a future direction, additional criteria representing pathologic differences in Akita mice will be developed. Also, each type of leukocytes; neutrophils, machrophages, B lymphocytes and T lymphocytes, will be detected in 18 weeks old diabetic vs wild type mice.

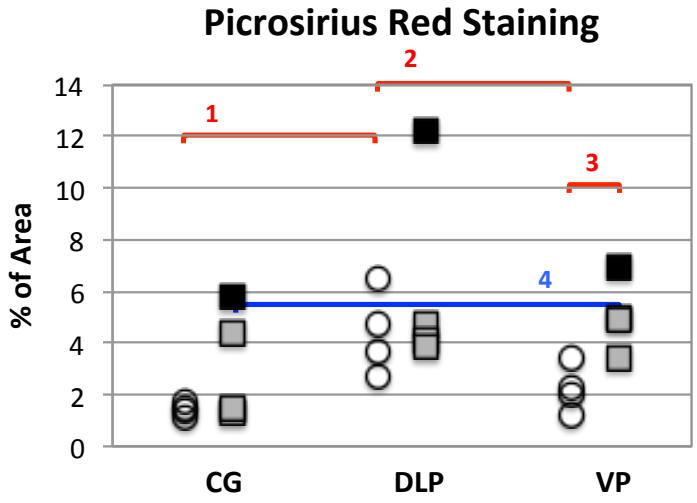

Figure 2. Area of Picrosirius Red Staining (%) in each prostatic lobe; coagulating gland(CG), dorsal lateral prostate (DLP) and ventral prostate (VP) of 18 weeks old wild type (n=4) and diabetic (n=4)mice. Open circle: wild type, gray square: diabetic, black square: diabetic with severe inflammation. P-values: 1=0.008; 2=0.045; 3=0.008; 4=0.042. (Red line: comparison includes all animals in each group, Blue line: comparison excludes one diabetic with severe inflammation)

Reference

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