Supplementary Material, Figure S2: ORS/DVR Mouse Phenotyping Service Summary Report

Project number: 5-12 Investigator(s): Dr. Blackstone Genetic manipulation: Female *Spg20-/-* and *Spg20+/+* (three each)

I. Body/Organ Weights:

The body weights and the following organ weights were obtained at the time of necropsy from the sixmice that were submitted for evaluation.

Body	Heart	Liver	Brain
Thymus	Spleen	Kidneys	

The mutant mice and the wild-type mice submitted for evaluation ranged from 73-days to 310-days of age. Comparisons of body weights and comparison of organ weights of the mutant KO mice to the wild type mice were made using t-Test (Microsoft Excel). I consider a P-value of less than 0.05 to be an important change. A summary of the organ weights and body weight data from the mutant mice and the wild-type mice taken from the data in the Excel spreadsheet file follows.

Results:

The average body weight of the mutant mice (27.75 gms) is more than the average body weight of the wild-type mice (24.25 gms) and the P-value is 0.39. The average brain weight of the mutant mice (0.49 gms) is equal to the average brain weight of the wild-type mice (0.49 gms) and the P-value is 0.91. The average thymus weight of the mutant mice (0.044 gms) is more than the average thymus weight of the wild-type mice (0.038 gms) and the P-value is 0.42. The average heart weight of the mutant mice (0.129 gms) is less than the average heart weight of the wild-type mice (0.132 gms) and the P-value is 0.80. The average spleen weight of the mutant mice (0.105 gms) is more than the average spleen weight of the mutant mice (1.312 gms) is more than the average spleen weight of the mutant mice (1.312 gms) is more than the average liver weight of the mutant mice (0.35 gms) is more than the average kidney weight of the mutant mice (0.31 gms) and the P-value is 0.34.

A difference is not detected between the mutant KO mice and the wild type mice in the average body weight, nor in any of the averaged organ weights.

II. Hematology (see attached Excel file for complete data including normal ranges):

All of the following hematological values were measured from bloodcollected at the time of necropsyfrom the six mice:

Hemoglobin	WBC count	Neutrophil count*	Eosinophil count*
Hematocrit	MCV	Lymphocyte count*	Basophil count*
RBC count	MCHC	Monocyte count*	Platelet count
MCH			

* = absolute values are reported (total cell counts per volume); WBC – white blood cells; MCV – mean cell volume; RBC – red blood cells; MCHC – mean corpuscular hemoglobin concentration; MCH – mean corpuscular hemoglobin

The individual test results for all animals are listed in the attached Excel file. The test results ranges that are considered normal for the equipment used in this laboratory are also listed in the Excel spread sheet. The test results between the two groups of mice were compared using a simple average of the data from the groups, compared to the normal range for this laboratory. All blood films (smears) were evaluated histologically as a cross check for the hematology results.

Results:

The averaged white blood cell count for the mutant mice is in the normal range and for the wild-type mice it is above the normal range for this laboratory.

The averaged neutrophil counts for both the mutant mice and the wild-type mice are in the normal range for this laboratory.

The averaged lymphocyte count for the mutant mice is in the normal range and for the wild-type mice it is above the normal range for this laboratory.

The averaged monocyte counts for both the mutant mice and the wild-type mice are in the normal range for this laboratory.

The averaged eosinophil counts for both the mutant mice and the wild-type mice are in the normal range for this laboratory.

The averaged basophil counts for both the mutant mice and the wild-type mice are in the normal range for this laboratory.

The averaged erythrocyte counts for both the mutant mice and the wild-type mice are in the normal range for this laboratory.

The averaged hemoglobin levels in both the mutant mice and the wild-type mice are in the normal range for this laboratory.

The averaged hematocrit levels for both the wild-type mice and the mutant mice are in the normal range for this laboratory.

The averaged platelet counts for both the mutant mice and the wild-type mice are in the normal range for this laboratory.

The averaged mean corpuscular volumes (MCV) in both the mutant mice and the wild-type mice are in the normal range for this laboratory.

The averaged mean corpuscular hemoglobin (MCH)values in both the mutant mice and the wild-type mice are slightly below the normal range for this laboratory.

The averaged mean corpuscular hemoglobin concentration (MCHC) value in both the mutant mice and the wild-type mice are in the normal range for this laboratory.

III. Serum Chemistries (see attached Excel file for complete data):

The following analytes were measured in the blood from six submitted mice:

Glucose	Calcium	AST	LDH
Cholesterol	BUN	Bilirubin, total	Albumin
ALT	Phosphorus, inorganic	Protein, total	Creatine kinase (CK)
ALK	Creatinine	Triglyceride	

The individual test results for the mutant mice and the wild type mice are listed in the attached Excel file. The test results ranges that are considered normal for the equipment used in this laboratory are also listed in the Excel spread sheet. The test results between the two groups of mice were compared using a simple average of the data from the groups, compared to the normal range for this laboratory.

Results:

All of the averaged serum chemistry test values for the mutant mice and the wild-type mice are within the normal range for this laboratory; and specifically the triglyceride and cholesterol levels are not elevated, and do not appear different between the mutant mice and the wild-type mice.

IV. Gross Diagnoses(see attached report for full list of findings):

Gross abnormal findings were not noted in any of the mice at necropsy.

V. Histopathologic Diagnoses (see attached report for full list of findings):

The attached report titled 'Gross and Histopathologic Diagnosis' contain a full listing of all the abnormal histologic findings and incidental histologic findings for each individual animal. If no abnormal findings were observed in a specific tissue then no comment was made about that tissue in the report. If a tissue was not available for evaluation, a notation was made in the report that the tissue was not available for evaluation. Also, 5 step sections of the skull containing the ears and 5 step sections containing various endocrine organs were evaluated histologically in each mouse. The following organs were examined microscopically in all mice, unless otherwise noted:

V. Histopathologic Diagnoses (continued):

Skin	Thyroid glands	Kidneys
Lymph nodes	Spleen	Skeletal muscle
Salivary glands	Liver	Peripheral nerves
Thymus	Pancreas	Brain
Trachea	GI tract (all levels)	Spinal Cord
Esophagus	Reproductive tract	Femur, tibia and knee joint
Lungs	Adrenal glands	Incisor teeth
Heart	Nasal mucosa and sinuses	Gall bladder
Ears	Urinary bladder	Bone marrow
Eyes	Harderian glands	Tongue
Pituitary gland	Humerus and shoulder joint	Vertebra and tail
Accessory sex glands	Cranial bones	Parathyroid glands

Histologic findings:

There are few histologic findings in the mutant mice and the wild-type mice. Some changes are present in both groups of animals. The histologic changes that are noted in tissues from the mutant mice and wild type mice are changes of limited pathological significance, and do not represent a difference between the mutant mice and the wild-type mice.

VI. Summary:

Body/Organ weights:

In a comparison of the body weights and the organ weights using t-Test (Microsoft Excel)a difference is not detected between the mutant mice and the wild type mice in the average body weight, nor any of the averaged organ weights.

Hematology:

Very few of the averaged hematology values are changed from the normal range for this laboratory; these changes are limited in scope, and of limited pathological significance. I do not believe any of these changes represent a difference between the mutant mice and the wild-type mice.

Serum Chemistry:

The averaged serum chemistry test values for the mutant mice and the wild-type mice are all within the normal range for this laboratory.

Gross anatomical changes:

Gross abnormal findings were not noted in any of the mice at necropsy.

VI. Summary (continued):

Histopathological findings:

There are few histologic findings in the mutant mice and the wild-type mice. Some changes are present in both groups of animals. The histologic changes that are noted in tissues from the mutant mice and wild type mice are changes of limited pathological significance, and do not represent a difference between the mutant mice and the wild-type mice.

VII. Conclusions:

I did not see changes that I believe represent a difference when comparing these two groups of mice.

Mark A Bryant, D.V.M. Diplomate, A.C.V.P. Pathology Section DRSB, DVR, ORS, NIH