

Aging Domain Protocols

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There are three endpoints to the screening of test class mice that will be aged for 18 months after birth:

1. Determine morbidity and mortality in mutant mice pedigrees up to 18 months of age.

a. Morbidity. Each mouse will be weighed every month up to 18 months. Any mouse with body weight $20\% \leq$ or \geq mean body weight for age-matched controls will be considered morbid, will cease being aged, and will be evaluated as described in point 3 (*vida infra*).

b. Mortality. Aged pedigrees will consist initially of eight mice, which will be monitored daily for death. In control mice, significant mortality is expected to accrue after 12 months and to reach approximately 25% at 18 months. All mice that die before 12 months of age will be evaluated *postmortem* as described in point 3. Fifty-percent mortality will be considered excessive. At this point, aging of the pedigree will stop and the remaining mice evaluated and described in points 2 and 3.

2. Screen for behavioral changes of neurological dysfunction in mutant mice pedigrees up to 18 months (and in some cases 28 months). To be evaluated are pedigrees that survive to 18 months of age or pedigrees that had aging terminated before 18 months according to criteria in point 1. Age-matched control mice and four test class mice of all pedigrees will be evaluated with the series of behavioral screens at UTK. The tests used will be the same as the behavioral core screens that were done at 2 months of age. The advantages of this approach are that it not only will allow us to compare aged mutant pedigrees with controls but also changes in performance within a pedigree from 2 to 18 months. Any pedigree identified as an outlier will be evaluated in point 3 (*vida infra*). Pedigrees that do not meet this criterion will not be evaluated further.

3. Determine pathological changes in the nervous system in mutant mouse pedigrees that display aberrant aging using standard stains and additional stains that specifically highlight CNS abnormalities. All mouse pedigrees that have been identified as showing aberrant aging in points 1 or 2 are evaluated for necropsy and tissue archiving. In addition, all pedigrees that have been held for

aging will be evaluated with the identical series of neurohistological stains carried out by the Neurohistology core to determine the consequences of aging on the full battery of cellular phenotypes assessed.

- a. Necropsy and archiving tissue. Two mice from each pedigree will be examined. Mice will be sacrificed by decapitation. Total body weight will be measured, the cranium opened, and the brain removed and divided into right and left halves by midsagittal section. The right half of the brain will be flash frozen in liquid nitrogen and stored at -80°C for possible future studies. For the left half of brain, an axial section through midbrain will separate telencephalon from rhombencephalon, and these two pieces will be weighed separately. The cerebral hemisphere will be divided into two pieces by an axial section from frontal pole to occipital pole. Both pieces of cerebral hemisphere and the half of rhombencephalon will be placed in a tissue cassette with the cut surface down. The spinal cord then will be removed and two axial cross-sections, one each from the cervical and lumbar spinal cord, will be placed in the same tissue cassette. Finally, skeletal muscle from the posterior compartment of the distal hind limb will be removed, a cross-section cut, and the tissue placed in the same tissue cassette (A). This first tissue cassette will then be sealed and fixed by immersion in 10% buffered formaldehyde (formalin). A limited necropsy will then be performed with the heart, lungs, liver, stomach, spleen, kidneys, and reproductive organs examined. A cross-section of each of these organs will be placed in tissue cassette B and fixed in formalin. Any abnormal structure or mass identified during necropsy will be placed in tissue cassette C and fixed in formalin. These tissue blocks will then be embedded in paraffin.
- b. Additional histopathologic examination. Histopathologic examination will consist of cutting 8- μ m sections from each paraffin-embedded tissue block and staining with hematoxylin and eosin (H&E), an excellent general screen for structural abnormalities. Tissue sections from cassette A also will be stained with luxol fast blue—periodic acid Schiff (LFB-PAS) to evaluate white matter (LFB) and the integrity of intraparenchymal blood vessels (PAS). Immunohistochemistry for GFAP and ubiquitin will be performed on tissue sections from cassette A. GFAP is an excellent method to localize reactive astrocytosis in the CNS. Reactive astrocytosis is a highly sensitive but nonspecific reaction to injury in the CNS. The anti- GFAP picture will confirm the Neurohistology analysis as well as provide comparisons with the neuropathological picture obtained with the other stains used to visualize pathogenesis in the aged tissue. Many neurodegenerative

diseases are associated with the accumulation of abnormal structures either in the neuropil, in neurons, or in glia. Most of these structures are excessively conjugated with ubiquitin. Thus, ubiquitin immunohistochemistry has become an excellent method to highlight abnormal inclusions that are characteristic of many neurodegenerative diseases.

4. Extended aging analysis to 28 months of age. Pedigrees that reach 28 months of age are examined through the following additional analyses:

- a. Blood triglyceride and cholesterol measurements. Blood triglyceride and cholesterol measurements will be performed under the supervision of Dr. Naima Moustaid-Moussa of the Nutrition Department at UT-Knoxville, using clinical chemistry instrumentation available at the University of Tennessee's College of Veterinary Medicine located on the same campus. These will be performed as the mice approach the age of 18 months and again as the mice reach 28 months.
- b. Insulin, leptin and IGF-1 measurements. These hormonal levels will be measured in serum at 18 months and again at 28 months; these measurements will also be done on young mice just entering the aging. These assays will be performed by Dr. Moustaid-Moussa's laboratory, which regularly perform Enzyme and radioimmunoassays (RIAs and ELISA's) for insulin and leptin. Corticosterone levels are measured by Dr. Jim Nelson (TX) and IGF by MECORE Laboratory at St. Joseph Hospital in ME (Dr. Rosen's lab). These assays, combined with the growth curves generated from body weight data, may serve to predict which pedigrees are likely to exhibit increased longevity. These "highlighted" pedigrees will be available for more detailed examination by experts in the biology of aging.
- c. MicroCT Analysis. MicroCT images of the aged mice will supply both bone density and body fat content measurements using software developed in house. A series of microCT images is taken from one mouse from each pedigree at 6-7 weeks, 18 months and 28 months old. This whole-body, three-dimensional series is taken in "scanning mode" (a seven-minute scan) at about 1 mm resolution and is assessed for skeletal or soft tissue abnormalities. Algorithms in development will also be implemented for deriving body fat content and bone density measurements taken from these same images. Any skeletal or soft tissue abnormalities or abnormal changes in bone density and fat content over time will trigger the scanning of additional mice from the pedigree.
- d. Hypothermia Challenge. Challenge to the ability to maintain core temperature will be used to screen for energy-balance mutations. For the cold-room challenge, mice are placed at 4°C, in their home cages for two

hours. Then, the rectal temperature of each mouse is taken with a rapid read digital thermometer; normal mouse body temperature is about 37°C. Normal mice maintain body temperature (measured rectally) quite nicely after two hours at 4°C, while even a drop of one degree within two hours is significant, and may indicate abnormalities and age dependent alterations in metabolism or storage of nutrients, neurological control of homeostasis, body composition, etc. Only those pedigrees showing 3 or 4 members with the abnormality would be flagged.

- e. Body Weight Measurements. Each mouse in the aging colony is currently weighed once per month, beginning at weaning age, and those data are available in our database. We would continue this weighing for the additional 10 months to 28 months of age if NIA chooses, and can develop statistics within the database to generate growth curves for each pedigree (or each mouse) beginning at 3 weeks of age.

All of the data from these assays are entered into our existing Mutrack database (<http://www.tnmouse.org/mutrack/>) along with the large data sets from other assays performed on these same mice at earlier ages.