Supplementary Data

Materials and Methods

Animals

Dogs were born and housed in an AAALAC-accredited centralized vivarium at SNBL USA and were genotyped by a PCR-based method as previously described.[9] All procedures were performed in accordance with the Guide for the Care and Use of Animals and approved by the Institutional Animal Care and Use Committee. Baseline neurological examinations, force testing, and gait analysis were performed at approximately 10 weeks of age by investigators blinded to genotype. Dogs were then examined at 13, 15, and 17 weeks of age. Ten XLMTM dogs were assessed at 10 weeks of age, and 4 of these dogs did not undergo additional experimental manipulation and were available for assessment at 13, 15, and 17 weeks of age. Six wild-type (WT) littermate dogs were assessed at 10, 13, 15, and 17 weeks of age.

Neurological assessments

Neurological assessments were performed by a board-certified veterinary neurologist (J.M.S.) and recorded on a neurological examination form (Fig. 1). Gait and attitude were observed first, followed by examinations for cranial nerve function, postural reactions, segmental spinal reflexes, cutaneous trunci response, perineal response, and the presence of any muscle atrophy. Gait analysis included observations on stride length, the ability to run and jump, and the presence of exercise intolerance or increased respiratory rate/effort following exercise. Attitude was graded as ''BAR'' (bright, alert, responsive), ''QAR'' (quiet, alert, responsive), or ''QDR'' (quiet, depressed, responsive). Results for most parameters (cranial nerve and postural reaction testing) were graded from 0 to 2, with ''0'' indicating an absent response, ''1'' indicating a decreased response, and ''2'' indicating a normal response. Segmental spinal reflexes were also potentially graded as ''3,'' indicating an increased response, or ''4,'' indicating a clonic response. Measurements such as muscle atrophy or certain aspects of gait abnormality (bunny hopping, short-strided gait) were described as mild to severe and graded on a scale of 0–4 (0 = none, 4 = severe). Dogs were also assessed for the presence or absence of a dropped jaw (ability to hold the jaw in a closed position; Fig. 2) and the ability to jump or climb over a low platform (''crate test'').

Based on the data for gait stride and character, degree of muscle atrophy and weakness, and presence of dropped jaw, a neurological assessment score was assigned to each examination for each animal based on predetermined criteria (for abbreviated criteria and approximate age at which XLMTM dogs displayed the various clinical signs, see Table 1). Reflex scores were incorporated into a separate reflex grading scheme. Because withdrawal reflexes were preserved until the latest stages of the disease and because patellar and cranial tibial reflexes were considered somewhat more difficult to perform and evaluate, reflex scores were not incorporated into the neurological assessment score.

A second independent observer without previous medical training underwent a training session on neurological scoring criteria, then interpreting the neurological examination. This observer was blinded to dog genotype for both of the following assessments: (1) the independent observer reviewed written findings from neurological examinations previously completed by the neurologist and independently assigned a neurological assessment score based on the predetermined criteria; (2) the independent observer received blank neurological examination forms and reviewed video recordings of the neurological examinations. Videotaping was instituted later in the study and complete videotaped examinations were only available for 5 dogs at 10 and 13 weeks. Thus, only the full set of videotaped results from the 15-week ($n = 9$; 4 XLMTM and 5 WT—one WT missing) and 17-week ($n = 10$; 4 XLMTM and 6 WT) examinations were analyzed. The neurological examination forms were completed by the independent observer based on interpretation of the videotaped neurological examination, using the predetermined scoring criteria.

Gait analysis

Trained handlers walked leashed dogs along an instrumented carpet at a self-selected pace (''GAITRite Electronic Walkway''; CIR Systems Inc.) for multiple passes as previously reported.[19] Video recordings were also simultaneously collected for later quality control. Trials were screened based on the consistency of gait speed and pattern, with only walks selected for analysis. Runs, trots, or gallops, as determined by walk pattern along the carpet or by video, were excluded. Spatiotemporal measures known to be affected by the disease, such as gait velocity (cm/s), step length (cm), and stride length (cm), were determined from the walks using specialized software (GAITFour version 4.1; CIR Systems Inc.).

Functional assessment of hindlimb strength

A method of measuring isometric torque frequency and eccentric contraction torque of the hindlimb in dogs has been described,[20] and results from XLMTM and wild-type dogs have been developed and published by our lab.[21,22] Briefly, with the dog under sevoflurane anesthesia, muscle force (torque) was measured by positioning the dog in a specially designed stereotactic frame so that the distal pelvic limb (paw) pushed (measured as extension force) or pulled (measured as flexion force) a foot plate (lever) attached to an ergometer. Tibial and peroneal branches of the sciatic nerve were stimulated percutaneously at the stifle to cause extension and flexion, respectively, of the tarsal joint. Measurements obtained for analysis included hind limb torque extension and hind limb torque flexion. Torque data (N-m) were normalized to body mass (kg).[20,21]

Statistical analysis

Methods applied for analysis included (1) correlation coefficients, scatter plots, and dot plots and (2) Bland–Altman plots and limits of agreement. Data are presented as means ± standard deviation with the range of values.

For the validity analyses, correlation coefficients were used to assess the strength of the linear association between the neurologist's assessment score and previously defined measures of gait (step length, velocity, and stride length) and hind limb strength (hind limb torque extension and hind limb torque flexion). Analyses were performed at 10 and 17 weeks. Scatter plots were also generated to visualize the relationship between the score and other measures, and to identify points with unusual values. Dot plots were created to compare the distribution of the neurological assessment score by genotype.

For the reliability analysis, scatter plots were created to visualize the relationship between different scoring methods (neurologist vs. second observer scoring written exams, neurologist vs. second observer scoring videotaped exams, and second observer scoring written exams vs. second observer scoring videotaped exams), and correlation coefficients quantified the strength of the linear association. In addition, Bland–Altman plots and limits of agreement were used to assess the degree to which two methods agreed.[23,24] Bland–Altman plots help to assess the extent of systematic difference between the two methods by comparing the mean difference to zero. They also indicate whether the difference between the two methods is larger for certain values of the scores and whether agreement is particularly poor in some cases. Limits of agreement are commonly provided with Bland–Altman plots; they indicate the level of agreement which we can expect in general, but should be interpreted cautiously when the sample size is small.