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Research report

Neurobehavioral deficits in the KIKO mouse model of Friedreich's ataxia

Marissa Z. McMackin, Chelsea K. Henderson, Gino A. Cortopassi*

Department of Molecular Biosciences, University of California, Davis, CA 95616, USA

HIGHLIGHTS

- Novel neurobehavioral endpoints were discovered for the KIKO mouse model of Friedreich's ataxia.
- KIKO mice have a decreased grip strength endurance time on inverted screen test.
- KIKO mice have increased threshold of peripheral sensitivity using Von Frey monofilaments, and.
- KIKO mice have ataxic gait parameters using Treadscan automated gait analysis.
- A translationally relevant phenotypic suite provides multiple potential therapeutic assessments for drug testing.

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ABSTRACT

Friedreich's Ataxia (FA) is a pediatric neurodegenerative disease whose clinical presentation includes ataxia, muscle weakness, and peripheral sensory neuropathy. The KIKO mouse is an animal model of FA with frataxin deficiency first described in 2002, but neurobehavioral deficits have never been described in this model. The identification of robust neurobehavioral deficits in KIKO mice could support the testing of drugs for FA, which currently has no approved therapy. We tested 13 neurobehavioral tasks to identify a robust KIKO phenotype: Open Field, Grip Strength Test(s), Cylinder, Skilled Forelimb Grasp Task(s), Treadmill Endurance, Locotronic Motor Coordination, Inverted Screen, Treadscan, and Von Frey. Of these, Inverted Screen, Treadscan and Von Frey produced significant neurobehavioral deficits at >8 months of age, and relate to the clinically relevant endpoints of muscle strength and endurance, gait ataxia, and peripheral insensitivity. Thus we identify robust phenotypic measures related to Friedreich's ataxia clinical endpoints which could be used to test effectiveness of potential drug therapy.

1. Introduction

Friedreich's Ataxia (FA) is an inherited pediatric disease in which the reduction of a single protein, frataxin, results in weakness, peripheral insensitivity and ataxia. FA is caused by a GAA repeat expansion (65 + GAA) in the first intron of chromosome 9q [9]. Frataxin deficiency in human FA patients results in deficits in dorsal root ganglion, spinocerebellar tracts, cerebellar dentate nucleus, ataxia, and decreased life expectancy [25].

The knock-in knock-out (KIKO) mouse model of FA was first described in 2002 [27], and is an appealing model of FA, in which frataxin deficiency resulting from GAA expansions is observed, as well as a FXN gene expression signature [12]. A complete knock-out model is not possible due to embryonic lethality [13], but the partial knock-out coupled with GAA repeat expansion (KIKO model) does reliable decrease frataxin expression in mice that can be easily bred in-house. However, no robust neurobehavioral deficits have been described that could be used as markers for urgently needed drug testing for this incurable disease [27,29]. Because of the appealing nature of the model, and the subtlety of the phenotype, 13 neurobehavioral assays were attempted to clarify which would describe frataxin-dependent consequences, and would hopefully relate to neurobehavioral deficits in FA patients.

KIKO *vs* Control abilities were tested for 13 neurobehavioral tasks in multiple categories: Open Field, Grip Strength (Push-pull, Cage Lid Hang, Wire Hang), Cylinder, Skilled Forelimb Grasp (Staircase, Capellini Grasp), Treadmill Endurance, Locotronic Motor Coordination, Inverted Screen, Treadscan Gait Analysis, Von Frey Peripheral Sensitivity. Of these, Inverted Screen, Treadscan and Von Frey produced significant neurobehavioral deficits which relate to

* Corresponding author.
E-mail address: gcortopassi@ucdavis.edu (G.A. Cortopassi).

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the clinically relevant endpoints of muscle strength and endurance, gait ataxia, and peripheral insensitivity.

2. Materials and methods

2.1. Animals

Mice were bred in the UC Davis laboratory colony until testing at 9 months of age. For *in vivo* experiments knock-in knock-out (KIKO) mice (*Jackson Laboratories #012329 B6.Cg-Fxn(tm1.1Pand) Fxn(tm1Mkn/J)*) and littermate wild type mice of both genders were tested on the same day. KIKO mice harbor one allele of the frataxin (GAA)230 expansion mutation (Fxntm1Pand) on one chromosome, and one allele of the frataxin exon 4-deleted mutation (Fxntm1Mkn) on the homologous chromosome (CureFA.org). Careful consideration was taken to clean all apparatuses between animals to eliminate social odor cues. Animals were nine months old and individually housed at the time of testing. Mice were maintained on a (12 h light/12 h dark cycle). All experiments were approved by the UC Davis Institutional Animal Care and Use Committee. All animals were given food and *water ad libitum*. Researchers were blinded to genotypes at the time of testing. The KIKO group consisted of 5 males and 15 females. The wild type littermate controls (WT/WT) consisted of 4 males, 11 females. Body weights between the two groups were not different, and therefore did not influence the phenotypic measurements.

2.2. Inverted screen test

A modified version of Kondziela's Inverted Screen Test [14] was used to assess muscle strength and endurance in the FA mice. The novel inverted screen apparatus constructed in our laboratory was a 24 in square grid of wire mesh consisting of 12 mm squares of 1 mm diameter wire. It is surrounded by a 30in deep wooden perimeter frame which prevented the mice from escaping the apparatus, and was raised 50 cm above a padded surface by sturdy wooden legs. The mouse was placed in the center of the wire mesh screen before the screen was rotated to an inverted position slowly over 3 s, with the mouse's head declining first. When the screen was stable and standing on all four legs, the timer was started. The time when the mouse fell off was noted, or the mouse was removed when the criterion time of 20 min maximum was reached [5].

2.3. Grip strength

The grip-strength device (Push/Pull Mechanical force gauge by Imada) is comprised of a bar connected to a force transducer. The device was placed flat and horizontally over the edge of on an elevated table. Mice were held by their tails and lowered towards the bar. Mice were tested individually, multiple times until pulling away from the device horizontally resulted in both front paws grasping firmly and resisting to the point of pulling the force transducer measurably. Force was measured in grams [35].

2.4. Von Frey

Animals were habituated to the elevated apparatus for one hour prior to baseline testing. Animals were isolated physically and visually in individual chambers (Ugo Basile Grid Platform). Peripheral sensitivity was assessed using Von Frey Nylon Monofilament Touch Test Sensory Evaluator (Stoelting) application to the plantar surface of the most visible and stable hind limb with no left or right preference. The pre-calibrated applicators were applied in ascending order until a behavioral response of lift, flinch, lick/paw investigation was noted. After detecting the first behavioral response, 5 additional consecutive applications of fibers to the plantar surface of the hind paws using the Up-Down Method [26] determined the threshold of sensitivity for mice.

2.5. Treadscan

For measurement of gait parameters mice were placed on a transparent treadmill belt (CleverySys Inc), which was slowly increased in rotation speed. Recording began after an animal reached 8 m/min at a steady walking speed. A high-speed camera mounted underneath the treadmill recorded the movements of all four paws using BcamCap Image Capture at a rate of 100 frames per second for 20 s. After a rest period in the apparatus the experiment was then repeated at a speed of 12 m/min. Automated footprint analysis of gait was conducted in batch mode on the videos using Treadscan gait analysis software for all available parameters of gait [3].

3. Results

KIKO *vs* Control abilities were tested at 9 months of age for 13 neurobehavioral tasks in 9 categories: Open Field, Grip Strength (Push-Pull, Cage Lid Hang, Wire Hang), Cylinder, Skilled Forelimb Grasp (Staircase, Capellini Grasp), Treadmill Endurance, Locotronic, Inverted Screen, Treadscan, and Von Frey. The most robust neurobehavioral tests of the FA phenotype in the KIKO mice ($p < 0.05$) were the Treadscan, Inverted Screen, and Von Frey in group sizes of 20 KIKO *vs*. 15 control.

3.1. Muscle strength and endurance tests

The Inverted Screen Test measures the muscle strength and endurance of all four mouse paws. Using an Inverted Screen (Fig. 1a) to test the muscle strength and endurance profile of the FA model mice illustrated that the KIKO mice ($n = 20$) have a significantly reduced endurance strength profile ($p=0.01$). Wild type mice ($n = 15$) were able to maintain their grip for an average of 448.5 s with an SEM = 70 s. KIKO mice ($n = 20$) were able to maintain their grip for an average of 254.6 s with an SEM = 80.87 s.

Using the Plethysmometer "Push/Pull" Meter (Fig. 1b) WT mice had an average forelimb strength of 107 g with an SEM = 2.51 g. KIKO mice had an average forelimb strength of 106 g with an SEM = 3 g. There was no measureable difference between the two groups ($p=0.4$).

3.2. Von Frey

The Von Frey nylon monofilaments measure peripheral sensitivity of the plantar surface of the hindpaws. The human FA homolog is a peripheral sensitivity defect that results in inability to effectively gauge foot pressure and decreases the plantar reflex response. In the Von Frey test, progressively thicker nylon monofilaments relating to increasing force in grams are applied to the hindpaw plantar surface, and the first behavioral response is recorded as threshold response. We saw that the KIKO mice have an average 0.8 g lower peripheral sensitivity (Fig. 2) as compared to age and gender matched littermate control mice ($p = 0.038$). A significantly higher force (g) is required to elicit a behavioral response ($p < 0.05$), and thus they have an increased threshold to detect the tactile stimulus.

3.3. Treadscan

Treadscan is an automated gait analysis program that is becoming the standard for ataxia, replacing Footprint Ink Track. Treadscan monitors ~50 parameters of gait for all four paws. Gait ataxia is the pathomnemonic feature of Friedreich's ataxia. We focused our