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

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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.

### Keywords

Friedreich's Ataxia; Neurobehavioral; ataxia; Frataxin; automated gait analysis; peripheral sensitivity; Von Frey; Inverted Screen

## 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 (Campuzano et al., 1996). Frataxin deficiency in human FA patients results in deficits in dorsal root ganglion, spinocerebellar tracts, cerebellar dentate nucleus, ataxia, and decreased life expectancy (Marmolino, 2011).



**Author Contribution**

M.Z.M. designed the research. M.Z.M. and C.K.H. performed the research. M.Z.M. analyzed the data. M.Z.M. and G.A.C. wrote the paper.

**Disclosure**

The authors have nothing to disclose.

The knock-in knock-out (KIKO) mouse model of FA was first described in 2002 (Miranda et al., 2002), 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 (Coppola et al., 2006). A complete knock-out model is not possible due to embryonic lethality (Cossée et al., 2000), 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 (Miranda et al., 2002; Perdomini, Hick, Puccio, & Pook, 2013). 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 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 (12h light/12hr 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 (Deacon, 2013) was used to assess muscle strength and endurance in the FA mice. The novel inverted screen apparatus constructed in our laboratory was a 24in square grid of wire mesh consisting of 12mm squares of 1mm diameter wire. It is surrounded by a 30in deep wooden perimeter frame which prevented the mice from escaping the apparatus, and was raised 50cm 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 sec, 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 20min maximum was reached (Bonetto, Andersson, & Waning, 2015).

### 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 (Toledo et al., 2016).

### 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 (McMackin et al., 2016) 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 seconds. 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 (Beare et al., 2009).

## 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 ($p<.05$) 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=.01). Wild type mice (n=15) were able to maintain their grip for an average of 448.5 seconds with an SEM=70 seconds. KIKO mice (n=20) were able to maintain their grip for an average of 254.6 seconds with an SEM=80.87 seconds.

Using the Plethysmometer “Push/Pull” Meter (Fig 1b) WT mice had an average forelimb strength of 107 grams with an SEM=2.51grams. KIKO mice had an average forelimb strength of 106 grams with an SEM=3grams. There was no measureable difference between the two groups (p=.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 0.8g lower peripheral sensitivity (Fig 2) as compared to age and gender matched littermate control mice (p=.038). A significantly higher force (g) is required to elicit a behavioral response (p<.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 analysis of Treadscan data in KIKO mice on gait differences relevant to FA, which include: Track Width (i.e. spacing of weigh-supporting limbs), the Foot Support Percentage (foot surface contact time), and both Regularity Index and Normal Step Sequence Number (measures of gait regularity).

At a speed of 8 m/min (Fig 3a) KIKO mice front limbs exhibited an increased Track Width (p<0.05), and there was a non significant increased Rear Limb Track width (Fig 3b). At a speed of 12 m/min the KIKO mice displayed a highly significantly decreased Stride Number Frequency for all four paws (Fig 4a). The KIKO mice also showed a significantly decreased Instantaneous Running Speed, and Overall Average Running Speed (Fig 4b). The KIKO mice showed classical ataxia phenotypic markers with a decreased Normal Step Sequence, and decreased Regularity Index as well (Fig 4c–d)

## 4. Discussion

Here we examined several neurobehavioral tasks of the KIKO mouse model of FA with known frataxin deficiency. We identified multiple neurobehavioral deficits in these KIKO

mice, and analogous deficits are also observed in human FA patients with frataxin deficiency. Since these deficits are the presumptive consequence of frataxin deficiency, and since they overlap with those observed in FA, they could be used as markers of therapeutic benefit in animal testing of potential FA therapeutics.

Patients who are diagnosed with FA are measured for disease progress using the Friedeich's Ataxia Rating Scale (FARA)(Fahey, Corben, Collins, Churchyard, & Delatycki, 2007) which includes ataxia ratings, daily living, and neurological measurements. For ataxia-specific measurements The International Cooperative Ataxia Rating Scale (ICARS) (Storey, Tuck, Hester, Hughes, & Churchyard, 2004) is more focused specifically on the postural, gait, and ataxia measurements. The Scale for Assessment and rating of ataxia (SARA) specifically tests patients with feet together because the narrow stance provides less stability (Schmitz-Hubsch et al., 2006). Our results of the automated gait analysis show that the KIKO mice have a decreased regularity, which could be a translatable marker for ataxia in FA mouse models.

The average age of onset for patients is 10 years old (Caron, Burns, Castro, & Iannaccone, 2015). An inverse correlation exists between GAA repeat length and age of onset, and between GAA repeat number and severity of disease manifestation (Filla et al., 1996). For the KIKO mice with a repeat length of ~250GAA, the onset would be expected to be later and more mild as compared to the more severe complete knock-out CRE model (Lim et al., 2007), which explains why we see the phenotype at 9 months of age. Improving neurobehavioral phenotypic assessments in these mice improves reliability of the model, as well as providing a multi-faceted representation of the pathology.

Other mouse models of Friedreich's ataxia have reported a variety of functional characterizations of the transgenic manipulations. The YG22R (190 GAA repeats) and Y47R (9 GAA repeats) transgenic lines have a single copy of the FXN yeast artificial chromosome (YAC) transgene, while the YGR (90 and 190 GAA repeats) has two copies (Anjomani Virmouni, Sandi, Al-Mahdawi, & Pook, 2014). The YG22R and YG8R mice did have a motor coordination phenotype in multiple tests by 12 months of age as compared to Y47R and B6, but an increased body weight may have contributed to those results and no phenotype was observed by the naked eye.

In the CRE-mediated elimination of FXN model the mice were injected with pHLC vector into the medulla of loxP [frda] mice, and a performance defecit was detected on Accelerated Rotorod within 4 weeks (Lim et al., 2007). Obvious neurological signs were not declared to be observed in the CRE model. The FXN/KO Mc mice (Sarsero, Holloway, Li, Finkelstein, & Ioannou, 2014) contain a 188 kb Bacterial Artificial Chromosome (BAC) clone (RP11-265B8) with a GAA sequence within the region of the FXN gene that undergoes expansion. No gross behavioral or phenotypic differences were noted in those mice, and no locomotor differences were noted at 12 months of age. By contrast, a mouse model for *dystonia musculorum* which is a disease that does have some similarity to the neurodegeneration profile of FA patients, results in a robust phenotype with an earlier onset that is visible to the naked eye (Lalonde, Marchetti, & Strazielle, 2005) (Tseng, Lu, & Chien, 2006).

### 4.1 Inverted screen

Friedreich's ataxia patients exhibit altered hand function, which is difficult to test using only isometric grip force control tasks (Hermsdorfer, Wessel, Mai, & Marquardt, 1994). Muscle strength assessed by the forelimb grip strength test has been shown to be decreased in the YG22 mouse from 9 months of age (Al-Mahdawi, 2006), but we were unable to see that effect in the KIKO mice of the same age. In a Cre-RE mouse model of FA the muscular strength was also equivalent to controls as measured by dynamometer (Simon et al., 2004). The inverted screen incorporates another aspect of the Friedreich's ataxia by testing endurance.

The inverted screen (Deacon, 2013) has been used in other fields of research for muscle strength and endurance profiling in mice. Muscle strength of forelimbs alone is often tested through grip strength devices, but the Inverted Screen Tests both the fore and hind limbs together (Contet, Rawlins, & Deacon, 2001). Assessment of an Fxn KO/Mck mutant mouse model showed altered hindlimb strength (Filali, Lalonde, Gérard, Coulombe, & Tremblay, 2015) as compared to controls. Friedreich's Ataxia is considered to be a mitochondrial disorder (Kaplan, 1999), and can be anticipated to result in a decreased endurance profile. It was our hypothesis that the KIKO mice would have a significantly decreased muscle strength and endurance profile. The results showed that the KIKO mice did have a significantly reduced grip time.

### 4.2 Von Frey

Von Frey was used to detect peripheral sensitivity in mice. The nylon monofilaments are most often used to test an increase in sensitivity as a result of allodynia or hyperalgesia. In states of increased nociceptive sensitivity sensory polyneuropathy is often measured as a decreased mechanical threshold (Huehnchen, Boehmerle, & Endres, 2013). The monofilaments offer incremental, small changes in force though, and can therefore be used to show small changes in sensitivity in either direction. The filaments themselves are considered innocuous mechanical stimuli and have been assessed for mechanosensitivity of receptive fields for wide dynamic range neurons using electrophysiological isolation of lumbar spinal cord in rodents, and application of the monofilaments to the plantar surface of the paw (Jinks & Carstens, 1998). Patients with FA experience loss gradual loss of light touch (Maring & Croarkin, 2007). Cell death of neurons in the dorsal root ganglion, as well as in the dorsal spinal cord, might be expected to alter peripheral mechanical sensitivity of the lower limbs, and in this mouse model we see that it does alter the peripheral sensitivity profile by a decrease in tactile sensitivity to mechanical stimulus on the plantar surface of the paw.

Von Frey was used as a novel endpoint to determine if the FXN mutation in the KIKO mice resulted in measureable peripheral sensitivity loss. FA results in a loss of large diameter neurons in the periphery, and decreased sensation in the lower limbs at the onset of the disease (Pandolfo, 2009). The primary sensory system has been classified into distinct neuronal types within the dorsal root ganglion of mouse lumbar DRGs (Usoskin et al., 2015), yet the translatable aspects of the sensory pathology associated with FA DRG remain to be clarified. Patients experience declining plantar reflex responses, and early

proprioceptive deficits (Borchers, Synofzik, Kiely, & Himmelbach, 2013). Patients also experience progressive tactile and nociceptive sensory loss associated with the degeneration of both large myelinated sensory nerve fibers and the dorsal column loss. It was our hypothesis that the KIKO mice would have altered peripheral sensitivity. The results showed that the KIKO mice had a significantly reduced sensitivity, as seen by an increased threshold of sensitivity to the tactile nylon monofilament stimulus.

### 4.3 Treadscan

The methods used to assess the mice for using Treadscan Gait Analysis were intended to mimic closely the methods of gait analysis used in clinical settings. The 8 m/min trial is a slow and easy pace for the mice, whereas the 12 m/min pace is an accelerated pace but not a full trot or run (personal observation). At 8 m/min speed the KIKO mice have a wider stance, a characteristic of cerebellar ataxia gait disorders (Sanders & Gillig).

Automated gait analysis provides multiple measures of gait that are relevant to the clinical ataxia rating scales such as the Friedreich Ataxia Rating Scale (Burk, Schulz, & Schulz, 2013). All four feet are measured individually in addition to cadence measurements, step patterns, and regularity indices. Gait abnormality observed in cerebellar ataxia includes a wide-base gait and decreased regularity in step sequence (“Pathology of the Aging Human Nervous System”, Page 330). Clinical research for Spinocerebellar Ataxia Type 6 (SCA6) has shown that increased stance width is associated with a decreased sway as compensation for the instability related to disrupted sensorimotor processing (Bunn, Marsden, Giunti, & Day, 2013). The Treadscan automated gait analysis allows the mouse models to be compared to the human clinical automated gait analysis tests such as the Walkway System, and the Calibrated Anatomical System Technique (CAST) (Benedetti, Catani, Leardini, Pignotti, & Giannini, 1998). It was our hypothesis that the KIKO mice at 9 months of age would have a translatable cerebellar ataxia-like profile using the automated treadscan analysis. The results of the automated gait analysis show that the KIKO mice have an increased Track Width at 8m/min, and multiple altered parameters of Stride Frequency and Regularity at 12m/min.

## 5. Conclusions

The use of the Inverted Screen, Von Frey, and Treadscan provide a non-terminal and translatable neurobehavioral suite that is relevant to clinical manifestations observed in Friedreich’s Ataxia patients. The KIKO mouse neurobehavioral phenotype is robust at 9 months of age and includes cerebellar ataxia, decreased peripheral sensitivity, and decreased motor strength and endurance. Presumably these tests could be used longitudinally in the KIKO mouse model and other FA mouse models to monitor neurodegenerative disease progression and therapeutic benefit.

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## 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

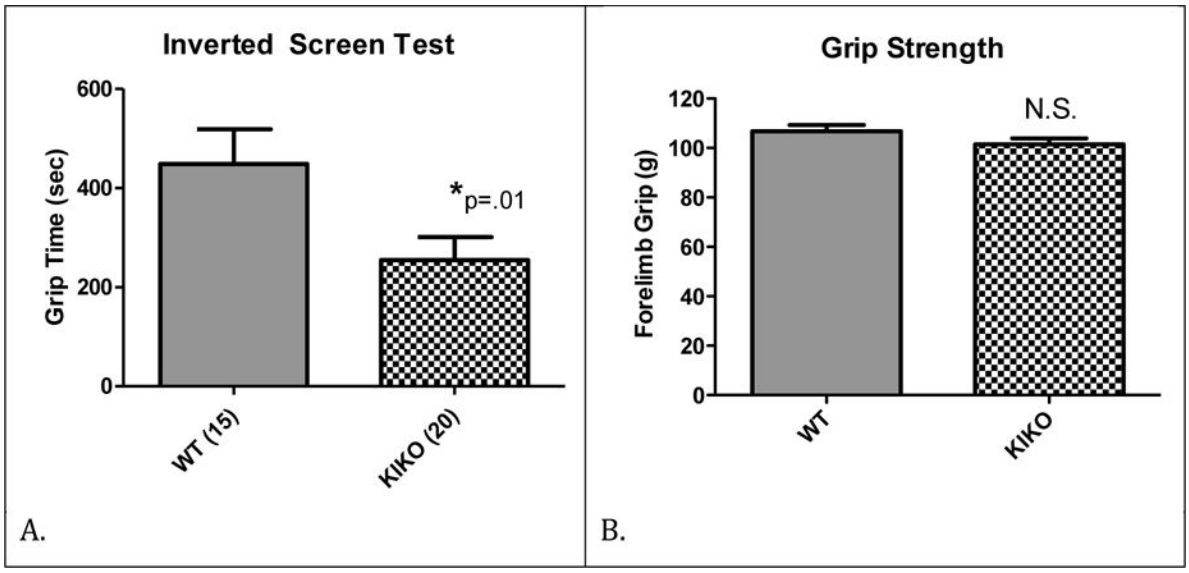


**Fig 1.**

(a–b). A. Inverted Screen testing of grip with both forelimb and hindlimb shows a significantly decreased strength and endurance profile for both male and female mice (p=. 01). B. Forelimb grip strength using a Push/Pull meter shows a non-significant difference between KIKO and WT littermate mice (p=.4).

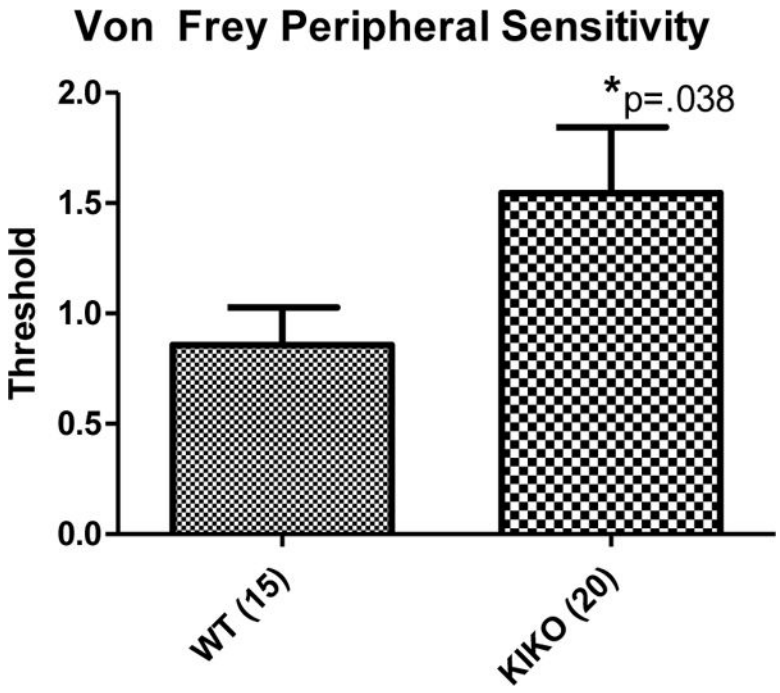


**Fig. 2.**

The threshold of sensitivity for the KIKO mice was increased as compared to WT controls (p=.038).

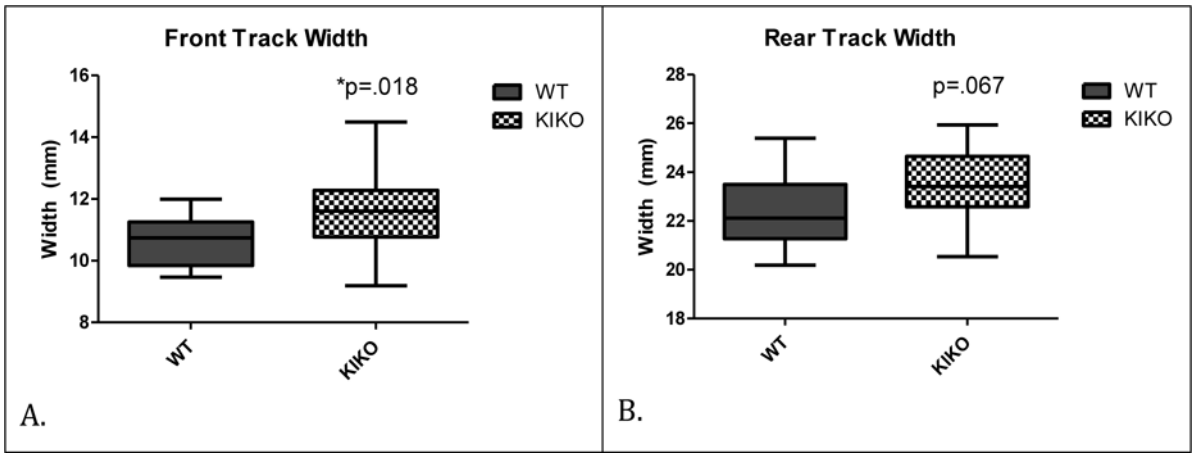


**Fig 3.**

(a–b). Treadscan Gait Analysis of KIKO and WT mice show altered foot measurements of steady rate "slow" walking (8m/min). Figures a&b show the distance measured between right and left paws to be increased in KIKO mice as compared to wild type.

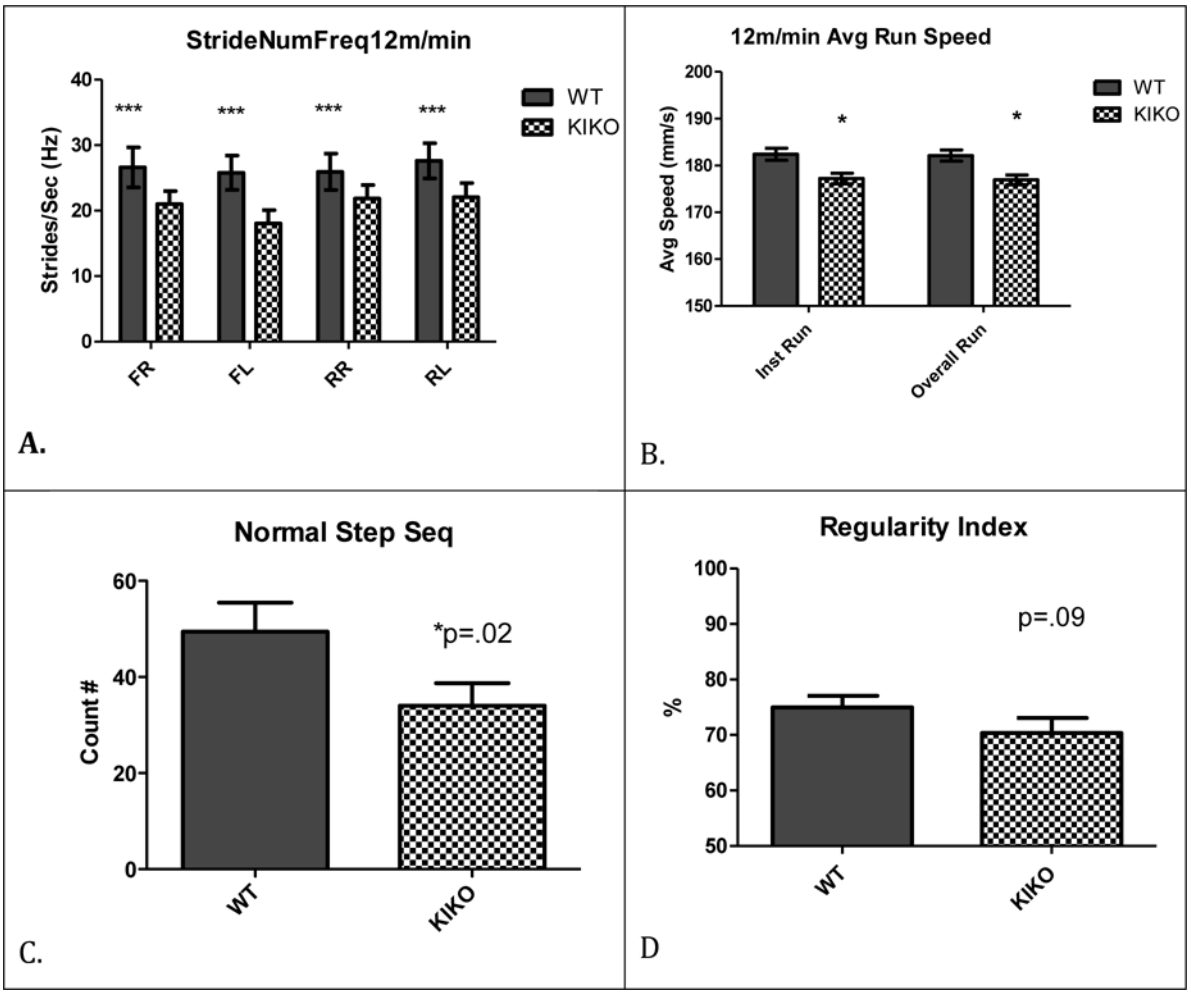


**Fig 4.**

(a–d) A. The average strides/sec (Stride Number Frequency) was decreased in KIKO mice for all four paws p=.0009. B. KIKO mice had both significantly decreased Overall and Instantaneous Running Speeds as compared to WT (p<.05). C–D. KIKO mice had reduced Normal Step Sequence, and Regularity Indices (p<.05).