Characterization of Glra1 spasmodic mouse to establish a neuromuscular contracture model for human neuromuscular diseases

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INTRODUCTION: Neuromuscular contractures (NC) are a prevalent (17M people worldwide) cause of joint deformity due to relative muscle-tendon unit shortening with decreased joint range of motion. NC occur in children suffering from a broad range of neuromuscular disorders (e.g. cerebral palsy & hyperekplexia) and lead to persistent disability [1]. We previously demonstrated postnatal NC formation following neonatal nerve transection; however, whether these differences are similarly seen in spastic NC remains unanswered given that currently there are no pre-clinical models of spastic NC. Here, we characterize a genetic model of NC formation in the mouse using the spasmodic mutant with loss of function in the glycine receptor 1 unit (*Glra1*) [2]. Remarkably, case reports of familial cohorts with conserved nonfunctional mutations in *Glra1* exhibit broad postnatal NC [3]. Therefore, we hypothesize that spasmodic *Glra1* mice will develop systemic joint contractures with resultant skeletal deformity and gait abnormalities consistent with NC in humans. Based on prior results demonstrating a role of myotendon elongation during NC formation, we further hypothesize that myotendon elongation also underlies NC formation in spastic *Glra1* mice [4] This study seeks to establish *Glra1* spasmodic mice as a reliable pre-clinical model of NC formation due to spastic neuromuscular disease. Development of this model in *Glra1* Hz mice will shed light on the cellular and molecular mechanisms that drive neuromuscular contracture formation.

METHODS: Postnatal day 120 (P120) *Glra1* Het (heterozygous) and *Glra1* Hz (homozygous) mice were subject to micro-CT, X-ray imaging, range of motion (ROM) tests, myotendon length measurement and gait analysis (total n=23 males + 11 females). At least 5 mice per genetic group were used for each experiment (see figures) with mixed sexes. Welch's t test was used for the study (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001). For range of motion testing, mice were euthanized and shortly after, limbs joint ROM was quantified with a fixed weight applied to tension joints equally. All animal studies were carried out under approved IACUC.

RESULTS: Consistent with clinical progression, *Glra1* Hz mice developed ankle equinus and skeletal deformity (hindfoot cavus and talar dome flattening) compared to the wild-type controls (*Glra1* Het) (**Fig. 1A**). Range of motion tests of the hindlimbs or upper extremities show decreased range of motion consistent with NC formation (**Fig. 1B & C**). Careful dissection of gastrocsoleus tendon/myotendon demonstrated myotendon elongation consistent with observation of NC formation following neonatal denervation (**Fig. 1D**) [4]. Gait analysis indicated that both forepaws and hindpaws of mutant mice exhibited abnormal gait patterns (altered center of mass, decreased coordination, and decreased range of motion) (**Fig. 2**).

DISCUSSION: Although previous studies showed that Glra1 mutant mice present with hypertonia, the more severe contracture phenotype was not comprehensively analyzed or reported. We recently showed in a surgical model of NC formation, development of myotendon elongation consistent with findings in *Glra1* Hz mice, suggesting there may be a conserved etiology of NC formation [4]. Here, we confirm that several features of NC in humans, decreased ROM and skeletal deformity are observed in mutant mice with functional consequences demonstrated by altered gait. In summary,, this study defined the phenotypes of *Glra1* spasmodic mice with limb abnormalities and a novel key feature of contracture. Ongoing studies will investigate hip and spine defects as well as molecular signaling defects in the myotendon to delineate systemic neuromuscular contractures.

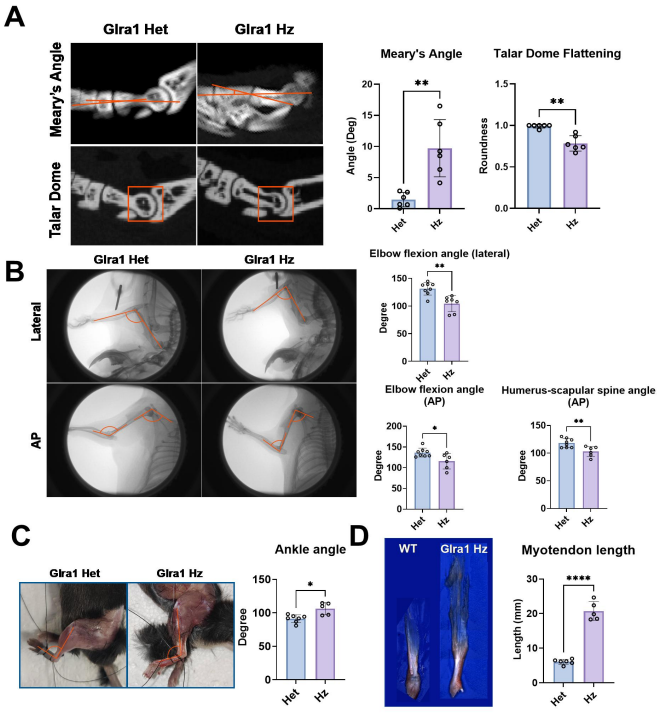

Fig. 1 *Glra1* homozygous mutation results in restricted range of motion along with defective bone and myotendon structures in P120 mice. A, Micro-CT of hindlimbs. (Het & Hz: n=6) **B**, X-ray imaging of upper extremity at anteroposterior (AP) and lateral positioning with weight loading on forepaws.(Het: n=8, Hz: n=7) **C**, Range of motion test of hindlimbs. (Het: n=7, Hz: n=5) **D**, Myotendon length (Het: n=6, Hz: n=5).

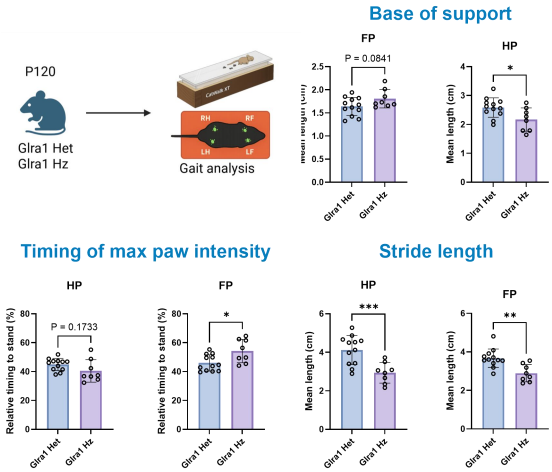

Fig. 2 *Glra1* homozygous mutation is associated with abnormal gait patterns for both forepaws and hindpaws. FP, forepaws; HP, hindpaws. (Het: n=12, Hz: n=8).

SIGNIFICANCE/CLINICAL RELEVANCE: Here we establish *Glra1* Hz mice as a novel pre-clinical model of spastic NC formation in the mouse. This platform would benefit further investigations of cellular and molecular mechanisms of neuromuscular contracture seen in multiple neuromuscular diseases such as hyperekplexia and cerebral palsy.

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