

News Release

Title

ECEL1/DINE mutation implicates impaired axonal arborization of motor nerves in the pathogenesis of distal arthrogryposis (DA)

Key Points

- √ ECEL1/DINE-deficient motor nerves indicated impaired axonal arborization in skeletal muscles in the forelimbs and hindlimbs.
- √ The axonal arborization defect in foot muscles appeared more severe than in other hindlimb muscles, which was partially consistent with the distal-proximal phenotypic discordance observed in DA patients.
- √ ECEL1/DINE knock-in mouse which carries the point mutation seen in DA patients showed similar axonal arborization defects.
- √ Abnormal arborization of motor axons and subsequent failure of NMJ formation could be a primary cause of DA with ECEL1/ DINE mutation.

Summary

Dr. Sumiko Kiryu-Seo and Prof. Hiroshi Kiyama, (Department of Functional anatomy & Neuroscience) in Nagoya University Graduate School of Medicine (Dean: Masahide Takahashi, M.D., Ph.D.), and their collaborators Dr. Kenichi Nagata and Dr. Takaomi Saido in RIKEN (President: Dr. Hiroshi Matsumoto) experimentally demonstrated that the membrane-bound metalloprotease endothelin-converting enzyme-like 1 (ECEL1 in human) / Damage-induced neuronal endopeptidase (DINE in rodent) is a causal gene of a specific type of distal arthrogryposis (DA). In contrast to most causal genes of DA, ECEL1/DINE is predominantly expressed in neuronal cells, suggesting a unique neurogenic pathogenesis in a subset of DA patients with ECEL1/DINE mutation. The present study analyzed developmental motor innervation and neuromuscular junction formation in limbs of the ECEL1/DINE-deficient mouse. Whole-mount immunostaining was performed in ECEL1/DINE-deficient limbs expressing motor neuron-specific GFP to visualize motor innervation throughout the limb. Although ECEL1/DINE-deficient motor nerves displayed normal trajectory patterns from the spinal cord to skeletal muscles, they indicated impaired axonal arborization in skeletal muscles in the forelimbs and hindlimbs. Systematic examination of motor innervation in over 10 different hindlimb muscles provided evidence that ECEL1/DINE gene disruption leads to insufficient arborization of motor nerves after arriving at the skeletal muscle. Interestingly, the axonal arborization defect in foot muscles appeared more severe than in other hindlimb muscles, which was partially consistent with the distal-proximal phenotypic discordance observed in DA patients. Additionally, the number of innervated neuromuscular junction was significantly reduced in the severely affected DINE-deficient muscle. Furthermore, we generated a ECEL1/DINE knock-in (KI) mouse model with a pathogenic mutation, which was recently identified in DA patients. Axonal arborization defects were clearly detected in motor nerves of the ECEL1/DINE KI limb, which was

identical to the ECEL1/DINE-deficient limb. Given that the encoded sequences, as well as ECEL1/DINE expression profiles, are highly conserved between mouse and human, abnormal arborization of motor axons and subsequent failure of NMJ formation could be a primary cause of DA with ECEL1/DINE gene mutation.

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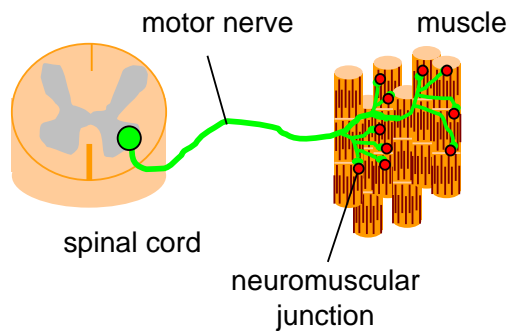
Research Background

Damage induced neuronal endopeptidase (DINE), a membrane-bound metalloprotease, has been identified as a nerve injury induced genes in analysis of rodent nerve regeneration mechanisms, and has found that the endothelin-converting enzyme-like 1 (ECEL1) whose function had been unknown at that time is a human ortholog of DINE. Recently, several independent groups reported a possibility that ECEL1/DINE is a responsible gene for type 5 distal arthrogryposis (DA), which is the most prevalent type of congenital-contraction disorder. In the present study we therefore attempted to determine the pathogenic mechanism of DA with ECEL1/DINE mutation via detailed morphological analyses of motor nerves in the DINE-deficient mouse, as well as in a newly generated ECEL1/DINE knock-in (KI) mouse with a pathogenic missense mutation of DA.

Research Results

Whereas all motor nerves innervating to individual muscle were detected in the ECEL1/DINE-deficient hindlimb, more precise observation revealed that motor nerve terminal branching was diminished in DINE-deficient muscles, and almost all fine branches were absent in all three muscles of the ECEL1/DINE-deficient foot. The axonal arborization defects in ECEL1/DINE-deficient mice were more severe at the most distal part of the limb compared with proximal regions. Concomitantly the number of neuromuscular junction (NMJ) is less in ECEL1/DINE-deficient mice. Since normal muscle development, including formation of the post-synaptic structure, occurred in ECEL1/DINE-deficient mice, the impaired formation of NMJ observed in ECEL1/DINE-deficiency was likely due to defects in the motor axon, not in the skeletal muscles. ECEL1/DINE knock-in mouse, which carries the point mutation seen in DA patients, showed similar axonal arborization defects. These results suggested that a human DA mutation likely affects ECEL1/DINE function, which strongly supports our hypothesis that axonal arborization defects are a major cause of DA with the ECEL1/DINE mutation.

Wild-type



DINE-KO or DINE-KI

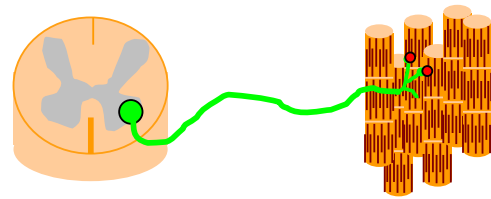


Figure: **Left panel**, During embryonic stages, motoneurons in spinal cord begin to extend their axons to their target muscles. When they reach the target muscles, they further extend into the muscles, and develop many elaborate branches within the muscles. **Right panel**, DINE-deficient mice exhibit abnormal intramuscular branching of motor nerves in the hindlimb. The human distal arthrogyrosis (DA) missense mutation knocked into the DINE locus also caused impaired intramuscular branching.

Research Summary and Future Perspective

Using two different ECEL1/DINE mutant mice, we found that ECEL1/DINE functional disruption leads to impaired axonal arborization of motor nerves in limb muscles. We also found that defective motor nerves lead to subsequent NMJ formation failure. These results provide the first evidence that DA with ECEL1/DINE mutation could result from neurogenic pathogenesis through the developmental defect of presynaptic motor nerves in limbs. Another important finding was that severity of motor innervation differed among ECEL1/DINE-deficient muscles. ECEL1/DINE-deficient motor nerves exhibited a more severe phenotype in foot muscles compared with other proximal hindlimb muscles, partially explaining the distal-proximal phenotypic discordance observed in DA patients. A further understanding of ECEL1/DINE function, including its endogenous substrates, would shed light on the intramuscular arborization mechanisms of motor axons, as well as further the development of novel therapeutic strategies for DA.

Publication

Kenichi Nagata*, Sumiko Kiryu-Seo, Hiromi Tamada, Fumi Okuyama-Uchimura, Hiroshi Kiyama*, Takaomi C Saido*, (*co-corresponding authors) (2016) ECEL1 mutation implicates impaired axonal arborization of motor nerves in the pathogenesis of distal arthrogyrosis, *Acta Neuropathologica* (March 8, 2016)

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