Two alleles of unc-52 locus disrupting potential cell-binding motif of UNC-52

Rachel Wilsey1,*, Sabrina Hodge1, Krysta Kenney1, Jacob Wahl3, Roshni Jaffery1, Avery Braun4, Zhongjiang Qiu1* and Myeongwoo Lee1*

1One Bear Place 97388, Department of Biology, Baylor University, Waco, TX 76798, U.S.A
2To whom correspondence should be addressed: Myeongwoo_Lee@baylor.edu
3These authors contributed equally.

Description

The unc-52 gene in Caenorhabditis elegans encodes for the protein UNC-52 and is a homolog of the mammalian gene Heparan Sulfate Proteoglycan 2 (HSPG2), which encodes for the protein perlecain (Rogalski et al., 1993; Mullen et al., 1999). HSPG2 is implicated in the human diseases Schwartz-Jampel Syndrome type 1 and Dyssegmental Dysplasia, Silverman-Handmaker type (Arikawa-Hirasawa et al., 2001; Stum et al., 2006). The mutated unc-52 gene expresses a phenotype for uncoordinated movement (“Unc”), which involves progressive paralysis and retarded sarcomere construction (Martinez et al., 2018). The UNC-52 protein is localized in the striated muscle dense bodies and the basement membrane, where it plays an important role in developmental processes such as cell adhesion, cell migration, and signal transduction (Kihira et al., 2012). Within the unc-52 gene, an RGD (Arg- Gly- Asp) sequence is located at amino acid locations 746, 747, and 748 in exon 7. Exon 7, containing RGD748, is included in all three major isoforms, short (S), medium (M), and long (L) (Mullen et al., 1999). The RGD sequence is part of the Laminin IV type A domain and primarily functions as a cell attachment and adhesion site for integrins (Rogalski et al., 1993; Mullen et al., 1999). In this study, two separate mutations were performed on this unc-52 RGD sequence using CRISPR-Cas9 technology. The unc-52(kq748) mutation replaced the aspartic acid (D) located at the 748 amino acid position with a glutamic acid (E) (Takahashi et al., 2007). The unc-52(kq745) mutation removed the RGD sequence by deleting amino acids 746, 747, and 748. Previous studies have shown that unc-52 gene mutations cause the disorganized distribution of pat-3 β integrin, which is a receptor for extracellular matrix proteins (Rogalski et al., 1995). In order to study the cellular phenotypes of the unc-52(kq748) mutation, a double mutant was created: pat-3::GFP; unc-52(kq748). Staining showed that localization of the pat-3::GFP reporter in the double RGD mutant appeared normal, with dense bodies and M-lines alternating along the muscle filaments (Figures 1A and 1B). In order to visualize the actin filaments in the body wall muscles of N2 wild type and unc-52(kq748) mutants, staining was performed using 0.4 U/mL rhodamine-conjugated phalloidin, Thermo Fisher Scientific, Waltham, MA (Figures 1C and 1D). This unc-52(kq748) staining showed no obvious abnormalities outside of interruptions due to fixation and slicing procedures (Figure 1D). Additional assays were performed to investigate any other physical or behavioral phenotypes.

Methods

CRISPR-Cas9 technology was used to create both the unc-52(kq748) and unc-52(kq745) mutations. For the unc-52(kq748) mutation, the DNA repair template (UNC52RGE748), crRNA (UNC52RGE748), tracrRNA (cat. #1072532), Cas9 nuclease (cat. #1081058), and a DNA repair template and all other DNA repair template and all other oligonucleotides were designed at IDT Inc., Coralville, IA. A double mutant was then created by crossing unc-52(kq748) II males with NKC358 pat-3::GFP III hermaphrodites. Homozygous double mutants from the F2 generation were identified as green-fluorescent progeny and confirmed through PCR genotyping.

Figure 1: A: Muscle cell of NKC358 pat-3::GFP animal. The dotted lines represent dense bodies (arrows) and straight lines represent M-lines (arrowheads); B: Muscle cell of a pat-3::GFP; unc-52(kq748) animal. The dotted lines represent dense bodies (arrows) and straight lines (arrowheads) represent M-lines. Localization appears similar to Figure 1A; C: Rhodamine-conjugated phalloidin staining of an N2 muscle cell. Actin cytoskeletons along the length of muscle are stained (arrows); D: Rhodamine-conjugated phalloidin staining of an unc-52(kq748) muscle cell. No obvious abnormalities in thin (actin) filaments (arrows) are present. Scale bar = 10 μm; E: Thrashing assay results for unc-52 (kq748) (1.4454 average thrashes/second, n=50), unc-52(kq745) (1.339 average thrashes/second, n=50), and N2 wild-type (1.99 average thrashes/second, n=50). * p-value < 0.05 compared to N2 wild-type.
(dpy-10) ZQDP10A GCUACCAUAGGCACCACGAG

Repair templates (5’ to 3’)

UNC52RGE748 caaaaatatgtgcaacctttcgcttcagTGCTCAATCAGCTACGCAAGACCGCCCACTCGGACTCCCG

UNC52RGD748D caaaaatatgtgcaacctttcgcttcagGTGTCAATCGACTACGCAAGATCAACTCGGACTCCCGCCAACCATA

Primers

UNC52RGE748SEQF TTCCTTGCTTCTGCTCAGGT

UNC52RGE748SEQR TGATCGGAGTTGCCATTTCCA

UNC52RGD748WTF TTCTATTGATTATGCTCGTGGGGAT

UNC52RGE748F TCAATCGACTACGCAAGAGGC

UNC52RGD748DF GTCAATCGACTACGCAAGATCAA

Reagents

BU748 unc-52(kq748) II, BU745 unc-52(kq745) II, and BU111 unc-52(kq748); pat-3::GFP are available upon request. NK358 pat-3::GFP III was purchased from Caenorhabditis Genetics Center, Minneapolis, MN.

Acknowledgments: These mutant lines were created during the course of BIO 4108 Cell and Developmental Biology Lab at Baylor University. Wild-type Bristol strain, N2, and fluorescence strains were purchased from the Caenorhabditis Genetics Center at the University of Minnesota, Minneapolis, MN.

References


Funding: Funding for BIO 4108 is provided by Baylor University.

Author Contributions: Rachel Wilsey: Writing - original draft, Formal analysis, Investigation. Sabrina Hodge: Investigation, Writing - review and editing. Krysta Kenney: Investigation, Writing - review and editing. Jacob Wahl: Investigation, Writing - review and editing. Avery Brau: Investigation, Writing - review and editing. Zhongqiang Qiu: Methodology, Validation, Investigation. Myeongwoo Lee: Writing - review and editing, Funding acquisition.

Reviewed By: Don Moerman

History: Received May 4, 2020 Revision received May 9, 2020 Accepted May 10, 2020 Published May 17, 2020

Copyright: © 2020 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Wilsey, R; Hodge, S; Kenney, K; Wahl, J; Jaffery, R; Brau, A; Qiu, Z; Lee, M (2020). Two alleles of unc-52 locus disrupting potential cell-binding motif of UNC-52. microPublication Biology. https://doi.org/10.17912/micropub.biology.000250