A null allele in the \textit{wdfy-3} selective autophagy gene of \textit{C. elegans}.

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Abstract

The \textit{C. elegans} WDFY-3 protein is important for cargo selection during selective autophagy and for regulating axon termination. The C-terminal region of WDFY-3 contains BEACH, WD repeats, and FYVE-like domains, all of which are required for selective autophagy. WDFY-3 also contains a large N-terminal region that is relatively uncharacterized. Currently, \textit{wdfy-3(ok912)} is the only mutant allele that has been characterized for this gene. This allele features a small deletion that is predicted to disrupt the C-terminal region of the protein. Here, we used CRISPR Cas9 to produce a new \textit{wdfy-3(cue30)} allele that is a near complete deletion of the coding region. We report that, unlike the existing \textit{wdfy-3(ok912)} allele, this new \textit{wdfy-3(cue30)} null allele causes a weak overextension phenotype in the PLM axon. Like the existing \textit{wdfy-3(ok912)} allele, the new \textit{wdfy-3(cue30)} null allele can suppress PLM axon termination defects caused by an \textit{fsn-1} null allele. Creating and characterizing new \textit{wdfy-3} alleles will increase our understanding of this gene and could help elucidate more of the gene's conserved functions.
Figure 1. A new deletion allele of wdfy-3 causes mild axon termination defects and suppresses axon termination defects caused by an fsn-1 null allele.

(A) Schematic of the predicted protein products for wildtype wdfy-3, wdfy-3(ok912) and wdfy-3(cue30). The wdfy-3(ok912) allele consists of a small deletion (marked by red bracket), insertion of GAGACA and resulting frameshift. Therefore, the BEACH, WD repeats, and FYVE-type domains are predicted to be non-functional in the protein product of the wdfy-3(ok912) mutant. The newly created wdfy-3(cue30) removes the first 33 of 36 exons and is predicted to be a null allele. Red arrows mark the cut sites of Cas9, with the base pair number relative to the start codon indicated above the cut site. (B) Quantification of PLM axon overextension defects in wildtype (wt), wdfy-3(ok912) and wdfy-3(cue30) animals. The null wdfy-3(cue30) animals showed increased penetrance of PLM axon overextension defects relative to wildtype and wdfy-3(ok912). (C) PLM axon overextension defects caused by the fsn-1(gk429) null mutation are suppressed by the wdfy-3(cue30) and wdfy-3(ok912) mutations. Axons were visualized with the muls32 transgene that encodes Pmec-7::gfp. Between 150 and 400 axons were observed in L4 stage hermaphrodites per genotype. Asterisks indicate statistically significant difference, Z-test for proportions (*p<0.01; ***p<0.0001). Error bars represent the standard error of the proportion.

Description

The human WDFY3 gene (also known as Alfy) encodes a scaffold protein that is required for selective autophagy and has been associated with autism (Clausen et al., 2010; De Rubeis et al., 2014; Filimonenko et al., 2010; Iossifov et al., 2012; Yuen et al., 2017; Wang et al., 2016). WDFY3 functions as an adaptor that links ubiquitinated cargo to the core autophagy components, thereby recruiting ubiquitinated targets into autophagosomes for eventual destruction in autophagolysosomes. Large scale genome sequencing studies have revealed a significant association between WDFY3 and autism (De Rubeis et al., 2014; Iossifov et al., 2012; Yuen et al., 2017; Wang et al., 2016). Consistent with this observation, WDFY3 is required for the normal development of axon tracts in mice (Dragich et al., 2016). Moreover, genetic analysis in C. elegans has indicated that wdfy-3 regulates axon targeting by functioning in a genetic pathway with egl-19, an ortholog of the CACNA1C autism-associated gene (Buddell et al., 2019). However, the mechanism through which WDFY-3 affects axon targeting remains mostly unknown.

As a first step in further exploring the role of WDFY-3 in axon targeting, we used CRISPR Cas9 to create the new wdfy-3(cue30) allele. This new wdfy-3(cue30) allele is a near complete deletion of the coding region of wdfy-3, except for the last three exons that make up the FYVE-type domain at the C-terminus of the protein (Figure 1A). This is unlike the previously studied loss-of-function wdfy-3(ok912) allele, which contains a small deletion that removes part of the BEACH domain in the C-terminal region (Figure 1A). The removal of the BEACH domain in the wdfy-3(ok912) allele also causes a frameshift mutation, likely resulting in the disruption of the WD repeats and FYVE-type domains (Kinchen et al., 2008). However, the large N-terminal region of WDFY-3 may not be affected by this mutation.

To determine how our new wdfy-3(cue30) allele affects axon targeting, we examined the PLM axon. The PLM cell body resides in the tail and extends an axon anteriorly along the lateral body wall. In wildtype worms, the PLM axon terminates at the midbody, prior to reaching the ALM cell body (Chalfie et al., 1985). We found that our new wdfy-3 deletion allele causes PLM axon termination defects that are significantly greater than those observed in wildtype (Figure 1B). Interestingly, the wdfy-3(ok912) allele does not cause PLM axon termination defects. Together, these observations suggest the possibility that the wdfy-3(ok912) allele might not be a null allele and that the uncharacterized N-terminal region of WDFY-3 could have functions important for axon guidance. Future investigations could test this hypothesis through the analysis of additional targeted deletions in wdfy-3.

Our prior work demonstrated that the wdfy-3(ok912) allele can suppress axon termination defects caused by an fsn-1 null allele (Buddell et al., 2019). To determine if our new wdfy-3(cue30) allele also has this property, we analyzed PLM axon termination in wdfy-3(cue30); fsn-1(null) double mutants. We found that wdfy-3(cue30) also suppresses overextension caused by fsn-1 (Figure 1C). However, this suppression did not bring the overextension penetrance back to wildtype levels like the wdfy-3(ok912) allele (Figure 1C). These observations are consistent with our previous report of a genetic interaction between wdfy-3 and /sn-1 (Buddell et al., 2019).

Methods

A CRISPR editing technique was used to create the wdfy-3(cue-30) mutation. Unique guide RNAs were designed to cut in two places to excise most of the coding region of the wdfy-3 gene without perturbing the function of nearby genes or the non-coding UTR region of wdfy-3. Guide RNAs used were upstream: GCGATTATGGATTATCTCG & downstream: AGTTTTGAACACGTGCGACG.
**C. elegans** strains were cultured and maintained on nematode growth medium (NGM)-agar plates using standard methods at 20°C (Brenner, 1974). Axons were labeled and observed as previously described (Xu & Quinn, 2012). Briefly, animals were mounted on a 5% agarose pad and observed with a 40x objective. PLM neurons were visualized with the *muIs32* transgene which encodes *Pmec-7::gfp + lin-15(+)* and is expressed in all mechanosensory neurons (Ch'ng et al., 2003).

**Reagents**

AGC52: *muIs32 [mec-7p::gfp + lin-15(+)] II; fsn-1(gk429) III*
AGC135: *muIs32 [mec-7p::gfp + lin-15(+)] II; wdfy-3(ok912) II*
AGC209: *muIs32 [mec-7p::gfp + lin-15(+)] II; wdfy-3(cue30) IV*
AGC259: *muIs32 [mec-7p::gfp + lin-15(+)] II; fsn-1(gk429) III; wdfy-3(ok912) IV*
AGC260: *muIs32 [mec-7p::gfp + lin-15(+)] II; fsn-1(gk429) III; wdfy-3(cue30) IV*

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**References**

Brenner S. 1974. The genetics of Caenorhabditis elegans. Genetics 77: 71-94. PubMed ID: 4366476

Buddell T, Friedman V, Drozd CJ, Quinn CC. 2019. An autism-causing calcium channel variant functions with selective autophagy to alter axon targeting and behavior. PLoS Genet 15: e1008488. PubMed ID: 31805042

Ch'ng Q, Williams L, Lie YS, Sym M, Whangbo J, Kenyon C. 2003. Identification of genes that regulate a left-right asymmetric neuronal migration in Caenorhabditis elegans. Genetics 164: 1355-67. PubMed ID: 12930745

Chalfie M, Sulston JE, White JG, Southgate E, Thomson JN, Brenner S. 1985. The neural circuit for touch sensitivity in Caenorhabditis elegans. J Neurosci 5: 956-64. PubMed ID: 3981252

Clausen TH, Lamark T, Isakson P, Finley K, Larsen KB, Brech A, et al.; Johansen T. 2010. p62/SQSTM1 and ALFY interact to facilitate the formation of p62 bodies/ALIS and their degradation by autophagy. Autophagy 6: 330-44. PubMed ID: 20168092

De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Ciccek AE, et al., Buxbaum JD. 2014. Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515: 209-15. PubMed ID: 25363760

Dragich JM, Kuwajima T, Hirose-Ikeda M, Yoon MS, Eenjes E, Bosco JR, et al., Yamamoto A. 2016. Autophagy linked FYVE (Alfy/WDFY3) is required for establishing neuronal connectivity in the mammalian brain. Elife 5:e14810. PubMed ID: 27648578

Filimonenko M, Isakson P, Finley KD, Anderson M, Jeong H, Melia TJ, et al.; Yamamoto A. 2010. The selective macroautophagic degradation of aggregated proteins requires the PI3P-binding protein Alfy. Mol Cell 38: 265-79. PubMed ID: 20417604

Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, et al., Wigler M. 2012. De novo gene disruptions in children on the autistic spectrum. Neuron 74: 285-99. PubMed ID: 22542183

Kinchen JM, Doukoumetzidis K, Almendinger J, Stergiou L, Tosello-Trampont A, Sifri CD, Hengartner MO, Ravichandran KS. 2008. A pathway for phagosome maturation during engulfment of apoptotic cells. Nat Cell Biol 10: 556-66. PubMed ID: 18425118

Wang T, Guo H, Xiong B, Stessman HA, Wu H, Coe BP, et al., Eichler EE. 2016. De novo genic mutations among a Chinese autism spectrum disorder cohort. Nat Commun 7: 13316. PubMed ID: 27824329

Xu Y, Quinn CC. 2012. MIG-10 functions with ABI-1 to mediate the UNC-6 and SLT-1 axon guidance signaling pathways. PLoS Genet 8: e1003054. PubMed ID: 23209429

Yuen RKC, Merico D, Bookman M, L Howe J, Thiruvahindrapuram B, Patel RV, et al., Scherer SW. 2017. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. Nat Neurosci 20: 602-611. PubMed ID: 28263302

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