Published in final edited form as:

J Inherit Metab Dis. 2017 March ; 40(2): 291–296. doi:10.1007/s10545-016-9986-1.

Flunarizine Rescues Reduced Lifespan in *CLN3* Triple Knock-Out *Caenorhabditis elegans* Model of Batten Disease

Young-Joon Kwon¹, Marni J. Falk^{1,2,*}, and Michael J. Bennett^{3,*}

¹Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA

²Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

³Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Summary

CLN3 disease (Spielmeyer-Vogt-Sjogren-Batten disease, previously known as classic juvenile neuronal ceroid lipofuscinosis, NCL) is a pediatric-onset progressive neurodegenerative disease characterized by progressive vision loss, seizures, loss of cognitive and motor function, and early death. While no precise biochemical mechanism or therapies are known, the pathogenesis of *CLN3* disease involves intracellular calcium accumulation that may trigger apoptosis. Our prior work in in vitro cell models of *CLN3* deficiency suggested that FDA-approved calcium channel antagonists may have therapeutic value. To further evaluate the potential efficacy of this approach in an otherwise untreatable disorder, we sought to compare the therapeutic effects and underlying mechanisms in an animal model of *CLN3* disease. Here, we used the well-characterized XT7 complete *cln-3* knockout strain of *C. elegans* to evaluate the therapeutic efficacy of calcium channel antagonist therapy in a living animal model of Batten disease. Therapeutic effects of five calcium channel antagonists were evaluated on XT7 animal lifespan and *in vivo* mitochondrial physiology. Remarkably, maximal therapeutic efficacy in this model animal was observed with 1 μ M flunarizine, the identical concentration previously identified in cell-based neuronal models of *CLN3* disease. Specifically, flunarizine rescued the short lifespan of XT7 worms and prevented their pathophysiologic mitochondrial accumulation. These results confirm the treatment efficacy and dosing of flunarizine in *cln-3* disease in a translational model organism. Clinical treatment

Corresponding Authors: Marni J. Falk, MD, ARC 1002C, 3615 Civic Center Boulevard, Philadelphia, PA 19104, Office. 215-590-4564; Fax. 267-426-2876, falkm@email.chop.edu and Michael J. Bennett, PhD, 34th and Civic Center Blvd, 5NW58, Philadelphia, PA 19104, Office. 215 590 3394; Fax 215 590 1998, bennettmi@email.chop.edu.

*Equal Contribution

Compliance with Ethics Guidelines.

Young-Joon Kwon, Marni J. Falk, and Michael J. Bennett declare that they have no relevant conflicts of interest.

Informed Consent and Animal Rights.

This article does not contain any studies with human or vertebrate animal subjects performed by the any of the authors.

Authors' Contributions.

MJF, YJK, and MJB conceived the experimental design. YJK performed the studies and analyzed the data. YJK, MJF, MJB wrote and evaluated the manuscript.

Consent for publication

All authors approved the final manuscript as submitted.

trials in *CLN3* human patients are now needed to test the dosing regimen and efficacy of flunarizine in individuals suffering with this otherwise untreatable and ultimately lethal neurologic disease.

Introduction

The neuronal ceroid-lipofuscinoses (NCLs) comprise a genetically heterogeneous group of progressive neurodegenerative, mostly autosomal recessive disorders for which no treatments exist. Some clinical trials are in progress, however, including the immunosuppressive drug mycophenolate, which has been shown to decrease neuroinflammation and improve motor performance in *Cln3*^{-/-} mice^{1,2} and for which a Phase 2 short-term clinical trial has been completed (NCT01399047; no results have yet been published), as well as enzyme replacement therapy in *CLN2* disease (NCT92678689; no results have yet been published). The NCLs are clinically characterized by variable onset of progressive vision loss leading to blindness, seizures, loss of motor and cognitive function, and premature death. Their shared histopathologic signature is neuronal cell death with accumulation of cytoplasmic autofluorescent storage material.³ *CLN3* disease (Spielmeyer-Vogt-Sjogren-Batten disease, previously known as classic juvenile NCL, OMIM 204200) is a pediatric-onset NCL caused by recessive mutations in *CLN3* located on chromosome 16. Most children with this disease have normal development until between 4 and 7 years of age, when they present with progressive visual loss, seizures, and relentless motor and cognitive decline ultimately leading to death in the mid to late 20s and early 30s.

Although the *CLN3* gene is well-characterized, the cellular pathway through which abnormal CLN3 protein leads to neuronal cell death is unclear.⁴⁻⁶ CLN3 protein (CLNP, Battenin) is a multi-spanning integral membrane protein that localizes in part to membrane lipid rafts. CLN3 protein functions to insert a double bond into the palmitate molecule of palmitoylated membrane-associated protein(s).^{7,8} However, no physiological protein partners for this palmitate desaturation or specific pathway(s) involved have yet been identified. Downstream of the desaturase effect, abnormal intracellular calcium accumulation may trigger apoptosis, a phenomenon which may be prevented by increased CLNP expression.⁹⁻¹⁴ Further, it appears that calcium-induced cytotoxicity that occurs with loss of CLNP function may be mediated by the neuronal calcium sensor calsenilin.¹⁵

Given the calcium induced cytotoxicity of this disorder, we previously screened known calcium-channel modulators for their ability to lower the calcium elevations that occur in *CLN3* knock down SH-SY5Y neuroblastoma cells. We identified several predominantly L-type calcium channel antagonists as candidate drugs to lower intracellular calcium levels in this model.¹⁶ Amlodipine further prevented apoptotic cell death in siRNA-inhibited *CLN3P* knockdown primary rat cortical neurons by normalizing elevated intracellular calcium levels.¹⁷ Thus, calcium channel blockers appeared to be promising therapeutic agents within two *in vitro* models of *CLN3* disease.

A CLN3 triple knockout *Caenorhabditis elegans* strain has previously been shown to serve as a translational animal model of Batten disease. Indeed, the nematode's simple nervous system and short lifespan (median lifespan of wild-type N2 Bristol strain is ~2 weeks) serves

as an intermediate model organism between yeast and mouse models.¹⁸ Three homologues of *CLN3* exist in *C. elegans* (*cln3-1*, *cln3-2*, *cln3-3*), each of which is encoded by a different gene and expressed at different life stages and anatomical locations.¹⁸ The *cln-3* triple knockout strain is named XT7 [*cln-3.2(gk41)I*; *cln-3.1(pk479)V*; *cln-3.3(gk118)V*] and was previously shown to have a shortened lifespan.¹⁸ Here, we used the XT7 complete *cln-3* knockout strain to evaluate the therapeutic efficacy of calcium channel antagonist therapy in a living animal model of Batten disease.

Methods

C. elegans strain selection

C. elegans strain, XT7, was studied that harbors deletion mutations in all three nuclear-encoded *cln-3* gene homologues [*cln-3.2(gk41)I*; *cln-3.1(pk479)V*; *cln-3.3(gk118)V*] (as was kindly provided by PEM Taschner).¹⁸ N2 Bristol was the wild-type control strain studied, which was obtained as a stable line from the *Caenorhabditis* Genetics Center (CGC, www.wormbase.org).

C. elegans lifespan analyses following calcium channel antagonist treatment during development and/or young adulthood

Animals were maintained in 20°C incubators. Synchronized nematode cultures were initiated by bleaching young adults to obtain eggs. Collected eggs were allowed to hatch overnight on 10 cm, unspread, Nematode Growth Media (NGM) plates, after which L1-arrested larvae were transferred to 10 cm NGM plates spread with OP50 *E. coli*, drug treatment, and/or appropriate DMSO-only buffer control.

Drug treatment was applied as follows: amlodipine, flunarizine, nicardipine, nifedipine, and nimodipine were each dissolved in 100% DMSO to a stock concentration of 1 mM and added to plates to achieve a final concentration on NGM plates of 1 µM, with a final DMSO concentration on NGM plates of 0.1%. For dose-curve lifespan studies with the lead candidate based on initial drug screen, flunarizine at concentrations of 10 µM, 1 µM, 0.5 µM, 0.1 µM was used, with final DMSO concentration on NGM plates of 1%. Worms were subsequently transferred to fresh NGM spread plates each week (i.e., weekly redosing occurred of drug or buffer-only control treatments to account for photosensitivity of calcium channel antagonists).

Upon reaching the first day of egg laying, synchronous young adult XT7 worms were moved to fresh 3.5 cm NGM plates seeded with OP50 bacteria and treated with a given drug. The same volume of water or DMSO was added to control animal plates. For purposes of higher-throughput lifespan screening, in order to remove the offspring of the nematodes under study from reaching adulthood, fluorodeoxyuridine (FUDR) was added to OP50-spread NGM plates to a final concentration of 100 mg/ml. Approximately 60 nematodes were studied per experimental condition, divided on three 3.5 cm NGM plates. Mortality was confirmed by stimulating nematodes lightly with a platinum wire; if the nematode did not move after stimulations, it was scored as dead and removed from the plate. Worms that died of

protruding/bursting vulva, bagging, or crawling off the agar were censored. Lifespan analyses were statistically analyzed in GraphPad Prism 5.0 as previously described.¹⁹

Relative fluorescence microscopy quantitation of calcium channel antagonist treatments on mitochondrial mass

Mitochondrial mass (MitoTracker Green FM, MTG) analyses were performed at 20°C using *in vivo* terminal pharyngeal bulb relative fluorescence quantitation, as previously described.^{20,21} Briefly, worms were treated with either 1 μ M flunarizine or 0.1% DMSO buffer control from the early development L1-larval stage. Upon reaching the first day of egg-laying, synchronous populations of young adults were moved to 3.5 cm NGM plates spread with OP50 *E. coli*, either 1 μ M flunarizine or 0.1% DMSO buffer control, and 10 μ M MitoTracker Green FM (to evaluate relative mitochondrial mass) for 24 hours. The next day, worms were transferred by worm picks onto 3.5 cm plates spread with OP50 *E. coli* without dye for one hour, to allow clearing of residual fluorescent dye from their gastrointestinal tract. Worms were then paralyzed *in situ* with 5 mg/ml levamisole. Photographs were taken in a darkened room at 160X magnification with a Cool Snap cf2 camera (Nikon, Melville, NY). A GFP2 filter set (Leica) was used for MitoTracker Green FM. Respective exposure time was 300 ms for MitoTracker Green FM. The resulting images were background subtracted, and the nematode terminal pharyngeal bulb was manually circled to obtain mean intensity of the region by using Fiji Is Just ImageJ.²² Fluorescence data for each strain were normalized to its same day control to account for day-to-day variation. A minimum of three independent experiments of approximately 50 animals per replicate were studied per strain per dye. Statistical analyses of the calculated difference in the mean fluorescence intensity between strains or under different experimental conditions were assessed by mixed-effect ANOVA using built-in code in MATLAB (Natick, MA, USA). This analysis was used to account for any potential batch effect due to samples being experimentally prepared, processed, and analyzed on different days, by including a batch random effect in the model. A statistical significance threshold was set at $p < 0.05$.

Results

Flunarizine [1 μ M] had the greatest effect of five calcium channel antagonists screened on rescuing the short lifespan of XT7 mutant worms

XT7 mutant worms were found to be significantly short-lived relative to wild-type (N2 Bristol) worms, consistent with prior report.¹⁸ Specifically, the XT7 strain had a median lifespan of 8.6 days, whereas wild-type N2 Bristol worms had a median lifespan of 11.2 days (23% reduction in XT7, Log-rank / Mantel-Cox test, $p = 0.002$). All five calcium channel antagonists tested led to an increase in the median lifespan, though only one drug, flunarizine, reached statistical significance based on the Log-rank Mantel-Cox test ($p < 0.05$). Flunarizine also led to the greatest increase in the XT7 strain in mean (10.3 days vs 11.8 days), median (8.6 days vs 10.4 days), and maximum (22 days vs 24 days) lifespan (Figure 1). Based on the results from the five drug screen on XT7 lifespan using the 1 μ M dose of each drug that was found to be effective in the *in vitro* cellular models (an Haack et al. 2011), flunarizine was selected for further dose effect studies of four concentrations. In 2 independent trials, 1 μ M flunarizine remained the optimum concentration to rescue XT7

mean, median, and maximum lifespan (Figure 2). Thus, these *in vivo* animal data support earlier *in vitro* studies suggesting that calcium channel antagonists have therapeutic efficacy to extend lifespan in this neurodegenerative disease.

Flunarizine reverses mitochondrial mass accumulation in day 1 young adult XT7 worms

Altered mitochondrial physiology has previously been implicated in the pathogenesis of CLN3 disease, with accumulation of subunit C of mitochondrial respiratory chain complex V.²³ While no antibody is available to test complex V accumulation in *C. elegans*, we sought to determine whether overall mitochondrial mass was altered in this model. Indeed, day 1 young adult XT7 mutant worms had a 10% increased mitochondrial mass ($p < 0.05$) as evaluated by fluorescence microscopy of relative mitotracker green fluorescence compared to wild-type N2 worms (Figure 3), suggestive that XT7 worms having mitochondrial proliferation. Remarkably, 1 μ M flunarizine treatment from development (i.e. L1 larval stages) prevented mitochondrial mass accumulation in this model (7.1% decrease from XT7 strain, $p < 0.05$) (Figure 3). Thus, these data support earlier reports of increased mitochondrial mass occurring in CLN3 disease, with this pathophysiologic aspect of the disease here prevented by 1 μ M flunarizine treatment.

Discussion

CLN3 disease is a progressive neurodegenerative disorder whose pathogenesis is incompletely understood and for which no therapies exist. Our studies described here in a validated XT7 animal model of CLN3-based Batten disease due to triple knockout of three *cln-3* homologous genes in *C. elegans* recapitulate both the short lifespan of the mutant strain and a novel indicator of its having abnormal mitochondrial proliferation. Most importantly, we show that the equivalent molar dose of a calcium channel antagonist, flunarizine (1 μ M), that was shown to normalized increased intracellular calcium levels in cellular neuronal model,¹⁶ effectively rescues the shortened lifespan of the XT7 *cln-3* triple gene knockout strain. It is possible that some other agents in this class could have additional physiologic benefit that was not evident on lifespan analysis with weekly redosing in the *C. elegans* model animal, as for example nifedipine is known to be unstable and subject to decomposition with photobleaching. However, we also show that 1 μ M flunarizine prevents mitochondrial accumulation in the XT7 strain. Thus, we conclude that flunarizine is a promising calcium channel antagonist to consider evaluating in human CLN3 disease.

Flunarizine's exact mechanism of action in improving cell and animal viability in CLN3 disease is not entirely clear. However, we postulate that flunarizine aids in the efflux into JNCL cells of calcium that is dysregulated as a downstream sequelae of the primary effect of deficient CLN3 protein, which involves failure of C16 desaturation of an unspecified *in vivo* target, and as we previously discovered in primary rat neurons, reduces neuronal apoptosis.¹⁷ The safety profile in humans of the calcium channel blockers used in these studies is well-known. The clinical indications for these agents have typically involved cardiovascular (arrhythmias, peripheral vascular disease) rather than neurodegenerative ones^{24–30}, although they have also been shown to have activity in central neurologic conditions such as migraines, vertigo, and as an adjunctive therapy for epilepsy.

Repurposing FDA approved drugs in rare diseases can improve outcomes; for example, the use of the anti-rheumatic agent auranofin was used in gastrointestinal stromal tumor (GIST).³¹ Additional studies are looking to repurpose FDA approved drugs in orphan disease treatments.^{32,33} Overall, we anticipate that these translational model animal studies to confirm treatment efficacy and optimize treatment dose of flunarizine in *cln-3* disease can be rapidly translated to clinical treatment trials in *CLN3* human patients suffering with this otherwise untreatable and lethal severe neurologic disease.

Acknowledgments

Funding

This work was funded in part by the National Institutes of Health (R01-HD065858 to MJF). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

We would like to thank P.E.M. Taschner for providing the XT7 strain used in this study.

References

1. Seehafer SS, Ramirez-Montealegre D, Wong AM, et al. Immunosuppression alters disease severity in juvenile Batten disease mice. *J Neuroimmunol.* 2011; 230(1–2):169–172. DOI: 10.1016/j.jneuroim.2010.08.024 [PubMed: 20937531]
2. Drack AV, Mullins RF, Pfeifer WL, Augustine EF, Stasheff SF, Hong SD. Immunosuppressive Treatment for Retinal Degeneration in Juvenile Neuronal Ceroid Lipofuscinosis (Juvenile Batten Disease). *Ophthalmic Genet.* 2015; 36(4):359–364. DOI: 10.3109/13816810.2014.886271 [PubMed: 24547931]
3. Haltia M. The neuronal ceroid-lipofuscinoses. *J Neuropathol Exp Neurol.* 2003; 62(1):1–13. [PubMed: 12528813]
4. Phillips SN, Benedict JW, Weimer JM, Pearce DA. CLN3, the protein associated with batten disease: structure, function and localization. *J Neurosci Res.* 2005; 79(5):573–583. DOI: 10.1002/jnr.20367 [PubMed: 15657902]
5. Rakheja D, Narayan SB, Bennett MJ. The function of CLN3P, the Batten disease protein. *Mol Genet Metab.* 2008; 93(3):269–274. DOI: 10.1016/j.ymgme.2008.01.001 [PubMed: 18688960]
6. Bennett MJ, Rakheja D. The neuronal ceroid-lipofuscinoses. *Dev Disabil Res Rev.* 2013; 17(3):254–259. DOI: 10.1002/ddrr.1118 [PubMed: 23798013]
7. Rakheja D, Narayan SB, Pastor JV, Bennett MJ. CLN3P, the Batten disease protein, localizes to membrane lipid rafts (detergent-resistant membranes). *Biochem Biophys Res Commun.* 2004; 317(4):988–991. DOI: 10.1016/j.bbrc.2004.03.146 [PubMed: 15094366]
8. Narayan SB, Rakheja D, Tan L, Pastor JV, Bennett MJ. CLN3P, the Batten's disease protein, is a novel palmitoyl-protein Delta-9 desaturase. *Ann Neurol.* 2006; 60(5):570–577. DOI: 10.1002/ana.20975 [PubMed: 17036287]
9. Lane SC, Jolly RD, Schmechel DE, Alroy J, Boustany RM. Apoptosis as the mechanism of neurodegeneration in Batten's disease. *J Neurochem.* 1996; 67(2):677–683. [PubMed: 8764595]
10. Puranam KL, Guo W-X, Qian W-H, Nikbakht K, Boustany R-M. CLN3 Defines a Novel Antiapoptotic Pathway Operative in Neurodegeneration and Mediated by Ceramide. *Mol Genet Metab.* 1999; 66(4):294–308. DOI: 10.1006/mgme.1999.2834 [PubMed: 10191118]
11. Dhar S, Bitting RL, Rylova SN, et al. Flupirtine blocks apoptosis in batten patient lymphoblasts and in human postmitotic CLN3- and CLN2-deficient neurons. *Ann Neurol.* 2002; 51(4):448–466. [PubMed: 11921051]
12. Persaud-Sawin D-ANW, VanDongen A, Boustany R-MN. Motifs within the CLN3 protein: modulation of cell growth rates and apoptosis. *Hum Mol Genet.* 2002; 11(18):2129–2142. [PubMed: 12189165]

13. Persaud-Sawin DA, Boustany R-MN. Cell death pathways in juvenile Batten disease. *Apoptosis*. 2005; 10(5):973–985. DOI: 10.1007/s10495-005-0733-6 [PubMed: 16151633]
14. Narayan SB, Rakheja D, Pastor JV, et al. Over-expression of CLN3P, the Batten disease protein, inhibits PANDER-induced apoptosis in neuroblastoma cells: Further evidence that CLN3P has anti-apoptotic properties. *Mol Genet Metab*. 2006; 88(2):178–183. DOI: 10.1016/j.ymgme.2006.01.011 [PubMed: 16515873]
15. Chang J-W, Choi H, Kim H-J, et al. Neuronal vulnerability of CLN3 deletion to calcium-induced cytotoxicity is mediated by calsenilin. *Hum Mol Genet*. 2007; 16(3):317–326. DOI: 10.1093/hmg/ddl466 [PubMed: 17189291]
16. An Haack K, Narayan SB, Li H, Warnock A, Tan L, Bennett MJ. Screening for calcium channel modulators in CLN3 siRNA knock down SH-SY5Y neuroblastoma cells reveals a significant decrease of intracellular calcium levels by selected L-type calcium channel blockers. *Biochim Biophys Acta*. 2011; 1810(2):186–191. DOI: 10.1016/j.bbagen.2010.09.004 [PubMed: 20933060]
17. Warnock A, Tan L, Li C, an Haack K, Narayan SB, Bennett MJ. Amlodipine prevents apoptotic cell death by correction of elevated intracellular calcium in a primary neuronal model of Batten disease (CLN3 disease). *Biochem Biophys Res Commun*. 2013; 436(4):645–649. DOI: 10.1016/j.bbrc.2013.04.113 [PubMed: 23769828]
18. de Voer G, van der Bent P, Rodrigues AJG, van Ommen G-JB, Peters DJM, Taschner PEM. Deletion of the *Caenorhabditis elegans* homologues of the CLN3 gene, involved in human juvenile neuronal ceroid lipofuscinosis, causes a mild progeric phenotype. *J Inher Metab Dis*. 2005; 28(6):1065–1080. DOI: 10.1007/s10545-005-0125-7 [PubMed: 16435200]
19. McCormack S, Polyak E, Ostrovsky J, et al. Pharmacologic targeting of sirtuin and PPAR signaling improves longevity and mitochondrial physiology in respiratory chain complex I mutant *Caenorhabditis elegans*. *Mitochondrion*. 2015; 22:45–59. DOI: 10.1016/j.mito.2015.02.005 [PubMed: 25744875]
20. Dingley S, Polyak E, Lightfoot R, et al. Mitochondrial respiratory chain dysfunction variably increases oxidant stress in *Caenorhabditis elegans*. *Mitochondrion*. 2010; 10(2):125–136. DOI: 10.1016/j.mito.2009.11.003 [PubMed: 19900588]
21. McCormack S, Polyak E, Ostrovsky J, et al. Pharmacologic targeting of sirtuin and PPAR signaling improves longevity and mitochondrial physiology in respiratory chain complex I mutant *Caenorhabditis elegans*. *Mitochondrion*. 2015; 22:45–59. DOI: 10.1016/j.mito.2015.02.005 [PubMed: 25744875]
22. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods*. 2012; 9(7):676–682. DOI: 10.1038/nmeth.2019 [PubMed: 22743772]
23. Palmer DN, Martinus RD, Cooper SM, Midwinter GG, Reid JC, Jolly RD. Ovine ceroid lipofuscinosis. The major lipopigment protein and the lipid-binding subunit of mitochondrial ATP synthase have the same NH₂-terminal sequence. *J Biol Chem*. 1989; 264(10):5736–5740. [PubMed: 2522438]
24. Elkayam U, Shotan A, Mehra A, Ostrzega E. Calcium channel blockers in heart failure. *J Am Coll Cardiol*. 1993; 22(4 Suppl A):139A–144A.
25. Elkayam U. Calcium channel blockers in heart failure. *Cardiology*. 1998; 89(Suppl 1):38–46. [PubMed: 9570428]
26. de Vries RJ, van Veldhuisen DJ, Dunselman PHJ. Efficacy and safety of calcium channel blockers in heart failure: Focus on recent trials with second-generation dihydropyridines. *Am Heart J*. 2000; 139(2):185–194. DOI: 10.1016/S0002-8703(00)90224-2 [PubMed: 10650289]
27. Trepakova ES, Dech SJ, Salata JJ. Flunarizine is a highly potent inhibitor of cardiac hERG potassium current. *J Cardiovasc Pharmacol*. 2006; 47(2):211–220. DOI: 10.1097/01.fjc.0000200810.18575.80 [PubMed: 16495758]
28. Cao K, Han F, Lin A, et al. Zhengtian Capsule versus flunarizine in patients with migraine: a multi-center, double-blind, double-dummy, randomized controlled, non-inferior clinical trial. *BMC Complement Altern Med*. 2016; 16:356.doi: 10.1186/s12906-016-1321-8 [PubMed: 27618916]
29. Lepcha A, Amalanathan S, Augustine AM, Tyagi AK, Balraj A. Flunarizine in the prophylaxis of migrainous vertigo: a randomized controlled trial. *Eur Arch Otorhinolaryngol*. 2014; 271(11):2931–2936. DOI: 10.1007/s00405-013-2786-4 [PubMed: 24166742]

30. Hasan M, Pulman J, Marson AG. Calcium antagonists as an add-on therapy for drug-resistant epilepsy. *Cochrane database Syst Rev.* 2013; (3):CD002750.doi: 10.1002/14651858.CD002750.pub2 [PubMed: 23543516]
31. Pessetto ZY, Weir SJ, Sethi G, Broward MA, Godwin AK. Drug repurposing for gastrointestinal stromal tumor. *Mol Cancer Ther.* 2013; 12(7):1299–1309. DOI: 10.1158/1535-7163.MCT-12-0968 [PubMed: 23657945]
32. Beaulieu CL, Samuels ME, Ekins S, et al. A generalizable pre-clinical research approach for orphan disease therapy. *Orphanet J Rare Dis.* 2012; 7:39.doi: 10.1186/1750-1172-7-39 [PubMed: 22704758]
33. Xu K, Coté TR. Database identifies FDA-approved drugs with potential to be repurposed for treatment of orphan diseases. *Brief Bioinform.* 2011; 12(4):341–345. DOI: 10.1093/bib/bbr006 [PubMed: 21357612]

Synopsis

Flunarizine treatment rescues the short lifespan and altered mitochondrial physiology in an established *C. elegans* model of Batten disease, a progressive neurodegenerative metabolic disorder for which no treatments are currently available to patients.

Figure 1.

XT7 animal lifespan screening of five calcium channel antagonists, which had shown to normalize increased intracellular calcium levels in rat neuronal models. Of five drugs screened, the 1 μ M dose of flunarizine was the most effective in rescuing mean, median, and maximum lifespans of XT7 strain toward that of wild-type N2 worms. N=60 worms per condition. *, $p < 0.05$; ***, $p < 0.001$ compared to XT7 strain.

Figure 2.

XT7 animal lifespan dose-span evaluation of flunarizine, which was the most effective calcium channel antagonist drug in rescuing the shortened lifespan of XT7 strain. 1 μ M dose of flunarizine remained the most effective dose in rescuing mean, median, and maximum lifespans of XT7 strain toward that of wild-type N2 worms. N=60 worms per condition. *, $p < 0.05$; ***, $p < 0.001$ compared to XT7 strain.

Figure 3. In vivo mitochondrial mass evaluation of flunarizine in XT7 animals

A) Representative image of *C. elegans* stained with MTG, where manual microscopic quantitation of relative MTG fluorescence intensity was limited to the 8-cell terminal pharyngeal bulb highlighted as the red circle. **B)** In day 1 young adults, XT7 mutant worms had 10% increased mitochondrial mass compared to wild-type N2 worms. 1 μ M flunarizine treatment initiated in early development from the L1 larval stage prevented the increased mitochondrial mass that occurs in XT7 worms (7.1% decrease from XT7 strain). Approximately 60 nematodes were studied per experimental condition, and each condition was conducted in biological triplicates (n=3). Bars and error bars convey mean $\pm$ SEM. *, $p < 0.05$.