EMBO Molecular Medicine

MYH3-associated distal arthrogryposis zebrafish model is normalized with para-aminoblebbistatin

Julia Whittle, Lilian Antunes, Mya Harris, Zachary Upshaw, Diane Sepich, Aaron Johnson, Mayssa Mokalled, Lilianna Solnica-Krezel, Matthew Dobbs, and Christina Gurnett
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Editor: Zeljko Durdevic

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

20th Apr 2020

Dear Prof. Gurnett,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the three referees who agreed to evaluate your manuscript. As you will see from the reports below, the referees acknowledge the interest of the study. However, they raise some concerns that should be addressed in a major revision of the present manuscript. Addressing the reviewers' concerns in full will be necessary for further considering the manuscript in our journal.

Acceptance of the manuscript will entail a second round of review. Please note that EMBO Molecular Medicine encourages a single round of revision only and therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. For this reason, and to save you from any frustrations in the end, I would strongly advise against returning an incomplete revision.

We realize that the current situation is exceptional on the account of the COVID-19/SARS-CoV-2 pandemic. Therefore, please let us know if you need more than three months to revise the manuscript.

I look forward to receiving your revised manuscript.

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

In the manuscript by Whittle et al. the authors use zebrafish to model a complex skeletal syndrome known as distal arthrogryposis (DA)which leads to joint contractures and scoliosis. DA is associated with mutations in the slow myosin heavy chain gene MYH3. However, how those mutations lead to the observed pathology is not well understood. The authors identified the zebrafish orthologue gene (smyhc1) and engineered both the equivalent of a common dominant mutation found in DA and a loss-of-function allele. Their analyses of these mutations implicate smyhc1 in the assembly of slow muscle fibers in the axial skeleton that lead to kinking of the notochord, resulting in vertebral fusions and severe spine defects. Interestingly, the authors were able to partially rescue the embryonic defects of the dominant mutation via transient inhibition of myosin activity.

Overall, this is an interesting manuscript that represents a good example of disease modeling in zebrafish as it investigates the effect of a pathogenic mutation in processes and structures that are relevant for the disease. It is also important to note that authors made proper use of currently available technologies to generate a genetically sound model. The result shown in this manuscript provide mechanistic insight into the origin of spine defects in DA and offer a potential therapeutic avenue for this type of disorders.

The manuscript is generally well written and requires only a few corrections. However, it would benefit from further elaboration and more precision in some areas (see below). If available, it would be useful to add data documenting some specific findings or assumptions.

Major points:

1-The authors report spine defects for the smyhc1-/- allele that develop between one two years of age. 1A-How common are those defects compared to that of WT or het siblings? 1B-given that defects occur in the caudal area and manifest in relatively old fish, a common occurrence in many genetic backgrounds, can those defects be tied to an embryonic defect that has no discernible phenotype early on? More data or a more cautious interpretation is needed.

Minor points:

2-Could the authors present RT data for the null allele?

3-Is there western blot data for the dominant mutation? hets would be most informative

4-Could the authors present or cite in situ hydridization data for smyhc1?

5-It would be useful to add a bit more information on the domain structure of MYH3/Smyhc1 and the basis of the identification of the orthologous mutation. Perhaps a multi-species alignment of the relevant domain could help.

Text issues:

6-In the intro the authors refer to the most common variant causing DA2A and DA2B is a R672C mutation, but then this is changed to R672H in the following paragraph, is this a typo?
7-The last sentence of the third section in Results, discussing the notochord "Despite the often severe..." is really difficult to read, please consider re-writing, perhaps splitting the sentence in two.

8-There is a typo "hmozygoteosus" in the discussion.

Experimental suggestion:
While not strictly necessary, there is one experiment worth performing should it be technically possible.

The authors use transient treatment with para-aminoblebbistatin to inhibit myosin activity, showing partial rescue of some of the most severe embryonic defects of the dominant allele. However, it is clear this treatment also has toxic side effects. Would it be possible to inject a cross of WT to smyhc1R673H/+ with a CRISPR pool targeting smyhc1? The expectation is that a number of fish

will effectively have a loss-of-function phenotype and mutations in cis to the dominant allele would rescue the most severe phenotypes, perhaps allowing the authors to examine whether spine defects can also be rescued.

Referee #2 (Remarks for Author):

The establishment of a zebra fish model for study of myosin mutations in skeletal muscle associated with congenital diseases is an important step, as vertebrate models are lacking. This is an important first step, however there are concerns that should be addressed to increase the potential impact of the study.

1. Blebbistatin compounds are known to be toxic, as pointed out by the authors. Indeed, in early stage muscle, these compounds have been shown to disrupt established sarcomeres and prevent sarcomere genesis. As such, it is unclear why these compounds were chosen. The Myokardia compound MYK-461 would appear to be a better choice, as it is less toxic and it, or it's analogs, are being developed for treatment of cardaic muscle disease in humans. There are also other myosin inhibitors that should be considered.

2. Because of the problem with the myosin inhibitors used, clear establishment of muscle contraction with MYH3 R672H as contributing to, or being causal for, malformation, dysfunction and lethality has not been established. More rigorous effort is required.

3. It is misleading to call MYH3 R673H a 'gain of function' mutation. This mutation has been demonstrated (Racca 2015) to impair relaxation in human muscle samples, and specific force of individual muscle fibers was reduced.

4. If the thought is that the MHY3 R673H mutation is hyper-contractile, an assessment of muscle function should be done. This would strengthen the argument. In addition, a quantitative analysis of sarcomere lengths should be performed. This should be easily accomplished, especially in conditions as those presented in Figure 6. Along with this, higher magnification analysis, perhaps with electron microscopy, could reveal features of altered sarcomere formation that would increase the ability to make mechanistic interpretations.

5. A more quantitative analysis of the features in Figure 5 may also be helpful.

Referee #3 (Remarks for Author):

This paper adopts a zebrafish model to examine the phenotypic consequences of an allelic series of mutations at the smyhc1 locus which is homologous to MYH3 in humans. A variety of phenotypes have been demonstrated in humans (caused by both biallelic and monoallelic genotypes) but the mechanistic relationship between them all remains obscure. This paper makes a useful contribution to this understanding in particularly noting the effects of adding null alleles in trans to a knock-in mutation R673H which has been recurrently shown to lead to a phenotype in humans characterised by a distal arthrogryposis and scoliosis. The authors characterise their phenotypes using conventional morphometry in addition to light microscopy. Finally they show that addition of a drug p-aminoblebbistatin prevents the development of one of the more severe phenotypes expressed in their experimental system.

The paper is well written, explains the concepts and rationale for the experiments clearly and presents the relevant data in a logical and straightforward fashion. This work certainly adds to the understanding on this perplexing range of conditions and their mechanistic basis in humans. I have two major suggestions to improve the manuscript.

1. Although the work sets out to examine the consequences in muscle and bone of these various genotypes, the exploration of the muscle pathology is stronger than that for bone, which is primarily confined to the gross morphological preps to demonstrate vertebral fusions. In mice, Zeiba et al have shown some evidence for a mechanistic basis for vertebral fusions in Myh3-/- mice that relates to re-direction of differentiation programmes in the intervertebral disks. Although some of the genotypes did (and some did not apparently, although I am not so convinced that authors can be categorical about this in what is depicted in Fig 1B; smyhc1-/-) show intervertebral fusions, linking these observations with those in the paper by Zeiba is not attempted. Some authors contend that Myh3 is expressed in bone and that the notochord phenotype is a cell intrinsic result of this, in contrast to it being a biomechanical result of myofiber induced forces on these structures. It would be very useful to have the authors address this issue.
It would be illuminating to understand if there is a mechanistic continuum across the genotypes examined here that is sponsored by the aberrations in TGF-beta (as shown by Zeiba et al) noted by them in this discussion. These data are particularly important in linking the work described here to the observation of a fusion phenotype in humans in DA8/SCTS.

2. The authors present some suggestive histological findings in muscle from their fish that suggests that the smyhc1R673H alleles lead to some disintegrity at the sarcomeric level (Fig 3A/B). This is intriguing because the genetics of the analogous conditions in humans does point to this allele being some kind of gain of function and the genetic data in this paper reinforce this contention. It would strengthen this paper if the anomalous structure of the sarcomere in these fish is teased out more. Some immunohistochemistry examining Z-disc structure for instance would be helpful as would stains to examine the hypothesis that the histological appearances might primarily result in cell death and degeneration of sarcomeric function in general. Presently, the current data stops at a frustratingly premature point!

Minor suggestions
1. Some of the linking conclusions presented in the results sections could be expressed more cautiously. For instance, the statement "We conclude that larval notochord abnormalities predispose smyhc1R673H/+ fish to vertebral fusions in adulthood" (pg 9) implies a causative link between two observations noted in this morphological series of observations where in fact this is only one possible conclusion from noting this association. Similarly, the last statement in the results section about the mode of action of p-aminoblebbistatin leaps straight to a mechanistic conclusion. Of course, this is one (very exciting) potential conclusion but a firm conclusion that this is the case is premature in the absence of experiments that would demonstrate that the effect of the drug is specific, obeys a dose response etc etc. I am not suggesting that these experiments need to be perform but more that the conclusions couched more cautiously.
p.14 homozygotesous fish
p15. The summary of the association of the human alleles that their associated phenotypes is not quite right, in part due to some shortcomings of the published literature. In all cases of SCTS caused by fully characterised bi-allelic genotypes, those genotypes have consisted of a haploinsufficient allele in trans with a hypomorphic loss of function allele. Dominantly inherited SCTS (and some sporadic cases) are always caused by missense alleles (likely conferring some kind of dominant negative effect). Some cases that appear to breach these rules only appear so because

they were incompletely characterised or the hypomorphic allele described by Cameron-Christie et al was not sought.

Reviewer's Comments to Author:

Referee #1

Comments to the Author:

In the manuscript by Whittle et al. the authors use zebrafish to model a complex skeletal syndrome known as distal arthrogryposis (DA) which leads to joint contractures and scoliosis. DA is associated with mutations in the slow myosin heavy chain gene MYH3. However, how those mutations lead to the observed pathology is not well understood. The authors identified the zebrafish orthologue gene (smyhc1) and engineered both the equivalent of a common dominant mutation found in DA and a loss-of-function allele. Their analyses of these mutations implicate smyhc1 in the assembly of slow muscle fibers in the axial skeleton that lead to kinking of the notochord, resulting in vertebral fusions and severe spine defects. Interestingly, the authors were able to partially rescue the embryonic defects of the dominant mutation via transient inhibition of myosin activity.

Overall, this is an interesting manuscript that represents a good example of disease modeling in zebrafish as it investigates the effect of a pathogenic mutation in processes and structures that are relevant for the disease. It is also important to note that authors made proper use of currently available technologies to generate a genetically sound model. The result shown in this manuscript provide mechanistic insight into the origin of spine defects in DA and offer a potential therapeutic avenue for this type of disorders.

The manuscript is generally well written and requires only a few corrections. However, it would benefit from further elaboration and more precision in some areas (see below). If available, it would be useful to add data documenting some specific findings or assumptions.

Major points:

1-The authors report spine defects for the smyhc1-/- allele that develop between one two years of age.
1A-How common are those defects compared to that of WT or het siblings?

Quantification of spinal defect prevalence have been added to the manuscript in Figure 4.

1B-given that defects occur in the caudal area and manifest in relatively old fish, a common occurrence in many genetic backgrounds, can those defects be tied to an embryonic defect that has no discernible phenotype early on? More data or a more cautious interpretation is needed.

We agree that this is a possibility- we also agree that this would be interesting to look at, but believe it is outside the scope of the paper. We have amended the text to a more explicitly cautious interpretation of the presented data.

Minor points:

2-Could the authors present RT data for the null allele?

We show that the smyhc1 protein is not present in smyhc1-/- fish by western Blot, and have added immunohistochemical data to Figure 1 showing its absence.

3-Is there western blot data for the dominant mutation? hets would be most informative

Due to the difficulty acquiring sufficient tissue from individually genotyped embryos for western blot for the dominant mutation, we have instead included immunohistochemistry data in Figure 1 that shows the presence of Smyhc1 protein staining within the muscles of smyhc1R673H/+ and smyhc1R673H/R673H fish.

4-Could the authors present or cite in situ hydridization data for smyhc1?

We added explicit reference to existing in situ hybridization data (Rauch et al., 2003).

5-It would be useful to add a bit more information on the domain structure of MYH3/Smyhc1 and the basis of the identification of the orthologous mutation. Perhaps a multi-species alignment of the relevant domain could help.

An alignment/domain schematic was added to Figure 1.

Text issues:

6-In the intro the authors refer to the most common variant causing DA2A and DA2B is a R672C mutation, but then this is changed to R672H in the following paragraph, is this a typo?

Thank you for catching this error, it has now been changed to reflect the prevalence of both variants.

7-The last sentence of the third section in Results, discussing the notochord "Despite the often severe..." is really difficult to read, please consider re-writing, perhaps splitting the sentence in two.

We have clarified the wording of this sentence.

8-There is a typo "hmozygoteosus" in the discussion.

Thank you for catching this. We have corrected the spelling.

Experimental suggestion:

While not strictly necessary, there is one experiment worth performing should it be technically possible.

The authors use transient treatment with para-aminoblebbistatin to inhibit myosin activity, showing partial rescue of some of the most severe embryonic defects of the dominant allele. However, it is clear this treatment also has toxic side effects. Would it be possible to inject a cross of WT to smyhc1R673H/+ with a CRISPR pool targeting smyhc1? The expectation is that a number of fish will effectively have a loss-of-function phenotype and mutations in cis to the dominant allele would rescue the most severe phenotypes, perhaps allowing the authors to examine whether spine defects can also be rescued.

This data can be acquired, and we agree that this experiment would be useful. However, we believe this experiment remains outside the scope of the current paper; we believe the experiment is worth exploring in future research endeavors.

Referee #2

Remarks for Author:

The establishment of a zebra fish model for study of myosin mutations in skeletal muscle associated with congenital diseases is an important step, as vertebrate models are lacking. This is an important first step, however there are concerns that should be addressed to increase the potential impact of the study.

1. Blebbistatin compounds are known to be toxic, as pointed out by the authors. Indeed, in early stage muscle, these compounds have been shown to disrupt established sarcomeres and prevent sarcomere genesis. As such, it is unclear why these compounds were chosen. The Myokardia compound MYK-461 would appear to be a better choice, as it is less toxic and it, or it's analogs, are being developed for treatment of cardaic muscle disease in humans. There are also other myosin inhibitors that should be considered.

Unfortunately, despite lengthy discussions with Myokardia, we were unable to receive any of this compound to evaluate in our model. Therefore, we proceeded with compounds which were readily accessible. Because blebbistatin compounds were previously demonstrated to be effective in zebrafish skeletal muscle by directly inhibiting actin-myosin interaction, we started with these. We agree that our model will be ideally suited to evaluate new molecules that have less toxicity as they are being developed.

2. Because of the problem with the myosin inhibitors used, clear establishment of muscle contraction with MYH3 R672H as contributing to, or being causal for, malformation, dysfunction and lethality has not been established. More rigorous effort is required.

We agree that a direct measurement of muscle tension would be very useful- however we believe this is outside the scope of our paper. To complement our manuscript, we have generated additional data, including sarcomere length, in our mutant fish to further characterize the effect of the variants on muscle function.

3. It is misleading to call MYH3 R673H a 'gain of function' mutation. This mutation has been demonstrated (Racca 2015) to impair relaxation in human muscle samples, and specific force of individual muscle fibers was reduced.

The wording of the mutation effect has been amended in the text to be more precise in its description in the third paragraph of the introduction.

4. If the thought is that the MHY3 R673H mutation is hyper-contractile, an assessment of muscle function should be done. This would strengthen the argument. In addition, a quantitative analysis of sarcomere lengths should be performed. This should be easily accomplished, especially in conditions as those presented in Figure 6. Along with this, higher magnification analysis, perhaps with electron microscopy, could reveal features of altered sarcomere formation that would increase the ability to make mechanistic interpretations.

We appreciate the suggestion, and have determined sarcomere lengths using higher magnification analysis and added this to Figure 6. We also performed additional DAPI and anti-α-actinin stains to further characterize the effect of the mutations on muscle in greater detail.

5. A more quantitative analysis of the features in Figure 5 may also be helpful.

Quantitative analysis of Figure 5A was conducted and data were added to Figure 5.

Referee #3

Remarks for Author:

This paper adopts a zebrafish model to examine the phenotypic consequences of an allelic series of mutations at the smyhc1 locus which is homologous to MYH3 in humans. A variety of phenotypes have been demonstrated in humans (caused by both biallelic and monoallelic genotypes) but the mechanistic relationship between them all remains obscure. This paper makes a useful contribution to this understanding in particularly noting the effects of adding null alleles in trans to a knock-in mutation R673H which has been recurrently shown to lead to a phenotype in humans characterised by a distal arthrogryposis and scoliosis. The authors characterise their phenotypes using conventional morphometry in addition to light microscopy. Finally they show that addition of a drug p-aminoblebbistatin prevents the development of one of the more severe phenotypes expressed in their experimental system.

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It would be illuminating to understand if there is a mechanistic continuum across the genotypes examined here that is sponsored by the aberrations in TGF-beta (as shown by Zeiba et al) noted by them in this discussion. These data are particularly important in linking the work described here to the observation of a fusion phenotype in humans in DA8/SCTS.

We share the reviewer's interest in the effects of these mutants on bone development. On closer review of the alizarin stained bone of our *smyhc1*$^{R673H/+}$ fish, it does appear that bone fusions are present even in regions where the spine is straight, suggesting that the variant may lead to fusions in the absence of notochord kinking. The discussion has been revised to reflect this.

2. The authors present some suggestive histological findings in muscle from their fish that suggests that the smyhc1R673H alleles lead to some disintegrity at the sarcomeric level (Fig 3A/B). This is intriguing because the genetics of the analogous conditions in humans does point to this allele being some kind of gain of function and the genetic data in this paper reinforce this contention. It would strengthen this paper if the anomalous structure of the sarcomere in these fish is teased out more. Some immunohistochemistry examining Z-disc structure for instance would be helpful as would stains to examine the hypothesis that the histological appearances might primarily result in cell death and degeneration of sarcomeric function in general. Presently, the current data stops at a frustratingly premature point!

We appreciate this suggestion. Therefore, we performed additional histology to evaluate the role of smyhc1 on muscle development, and after staining for α-actinin, found that smyhc1 is required for the proper development of the Z-disc (Figure 6C,D). Indeed, as the reviewer smartly predicted, cell death does appear to be involved in the histological appearance of the *smyhc1*$^{R673H/+}$ and *smyhc1*$^{R673H/+}$ muscle, as shown by Caspase-3 staining (Figure EV4).

Minor suggestions

1. Some of the linking conclusions presented in the results sections could be expressed more cautiously. For instance, the statement "We conclude that larval notochord abnormalities predispose smyhc1R673H/+ fish to vertebral fusions in adulthood" (pg 9) implies a causative link between two observations noted in this morphological series of observations where in fact this is only one possible conclusion from noting this association. Similarly, the last statement in the results section about the mode of action of p-aminoblebbistatin leaps straight to a mechanistic conclusion. Of course, this is one (very exciting) potential conclusion but a firm conclusion that this is the case is premature in the absence of experiments that would demonstrate that the effect of the drug is specific, obeys a dose response etc etc. I am not suggesting that these experiments need to be perform but more that the conclusions couched more cautiously.

The wording of these conclusions have been modified to yield a more cautious interpretation of the results.

p.14 homozygotesous fish

Thank you for catching this, the spelling has been corrected.

p15. The summary of the association of the human alleles that their associated phenotypes is not quite right, in part due to some shortcomings of the published literature. In all cases of SCTS caused by fully characterised bi-allelic genotypes, those genotypes have consisted of a haploinsufficient allele in trans with a hypomorphic loss of function allele. Dominantly inherited SCTS (and some sporadic cases) are always caused by missense alleles (likely conferring some kind of dominant negative effect). Some cases that appear to breach these rules only appear so because they were incompletely characterised or the hypomorphic allele described by Cameron-Christie et al was not sought.

Thank you for pointing this out, description of the literature has been amended to more reflective of previous research.

1st Revision - Editorial Decision 26th Aug 2020

26th Aug 2020

Dear Prof. Gurnett,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. As you will see the reviewers are now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

1) Please discuss the possible toxic effects of blebbistatin and how this may have influenced results of the study as suggested by the referee #2.

***** Reviewer's comments *****

Referee #2 (Comments on Novelty/Model System for Author):

The study would be improved by measurements of muscle contraction, this was suggested in the initial review. It is OK without, but impact is not as high as it could be.

Referee #2 (Remarks for Author):

The authors should discuss the possible toxic effects of blebbistatin and how this may have influenced results

Referee #3 (Comments on Novelty/Model System for Author):

The model addresses mechanism for these alleles that have not yet been addressed in a vertebrate. The muscle phenotypes are addressed well and the skeletal phenotypes are described but not mechanistically dissected

Referee #3 (Remarks for Author):

My concerns have been addressed adequately.

Editor's Response

1) Please discuss the possible toxic effects of blebbistatin and how this may have influenced results of the study as suggested by the referee #2.

This has been added to the manuscript in the results section.

Reviewer's comments

Referee #2 (Comments on Novelty/Model System for Author):

The study would be improved by measurements of muscle contraction, this was suggested in the initial review. It is OK without, but impact is not as high as it could be.

Thank you for the comment. We will work to study muscle contraction in future publications.

Referee #2 (Remarks for Author):

The authors should discuss the possible toxic effects of blebbistatin and how this may have influenced results

This has been added to the results section.

Referee #3 (Comments on Novelty/Model System for Author):

The model addresses mechanism for these alleles that have not yet been addressed in a vertebrate. The muscle phenotypes are addressed well and the skeletal phenotypes are described but not mechanistically dissected

Thank you for the comment. We are working on exploring the skeletal effects further in future publications.

Referee #3 (Remarks for Author):

My concerns have been addressed adequately.

2nd Revision - Editorial Decision 9th Sep 2020

The authors performed the requested changes.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Christina Gurnett
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2020-12356-V3

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- ➔ the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ➔ figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- ➔ graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- ➔ if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- ➔ Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- ➔ a specification of the experimental system investigated (eg cell line, species name).
- ➔ the assay(s) and method(s) used to carry out the reported observations and measurements
- ➔ an explicit mention of the biological and chemical entity(ies) that are being measured.
- ➔ an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- ➔ the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- ➔ a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- ➔ a statement of how many times the experiment shown was independently replicated in the laboratory.
- ➔ definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was determined based on experience from previous findings and animal availability, i.e., no power calculation was done prior to study design.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Depending on the nature and complexity of the assay, the maximum number of animals that could be tested were used to increase statistical power as much as possible.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples were excluded from analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Animals were randomly chosen for para-aminoblebbistatin treatment and genotype was determined after th experiment.
For animal studies, include a statement about randomization even if no randomization was used.	Animals were randomly chosen for para-aminoblebbistatin treatment and genotype was determined after th experiment.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Zebrafish were chosen at random as subjects before genotype was determined. When determining survivability of zebrafish genotypes, entire clutches were genotyped to avoid selective bias.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Individual zebrafish were chosen and photographed before genotype was determined. The investigator was not blinded before assessing skeletal, muscular, or gross anatomical abnormalities of zebrafish.
5. For every figure, are statistical tests justified as appropriate?	Wilcoxon-rank sum tests were used to establish a significant difference between 2 data sets, appropriate for the conclusions presented in the figures.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Non-parametric tests (Wilcoxon rank-sum test) were used to determine differences in sample populations, so normal distribution was not assumed in the statistical tests.
Is there an estimate of variation within each group of data?	An estimate of variaion was not included in the statistical analysis.

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Variance seems to vary between compared groups.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	F59: https://www.citeab.com/antibodies/150201-f59-myosin-heavy-chain-all-fast-isoforms anti-a-actinin: Clone EA-53, Sigma-Aldrich A7811 anti-Caspase 3: Sigma-Aldrich AB3623 anti-phospho-Smad2: Sigma-Aldrich AB3849
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Danio rerio (Zebrafish); strain AB, strain stl583 (R673H amino acid substitution in the gene smyhc1), strain stl582 (7 base pair deletion in the gene smyhc1);
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Experiments involving zebrafish are in compliance with the Gurnett lab protocol approved by IACUC (American Association for Laboratory Animal Science) at Washington University.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We have consulted and conformed to the ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No.