Eur J Transl Myol 31 (3): 10012, 2021 doi: 10.4081/ejtm.2021.10012

A revised model for mitochondrial dysfunction in Duchenne muscular dystrophy

Ai Vu Hong (1,2), Mathilde Sanson (1,2), Isabelle Richard (1,2), David Israeli (1,2)

(1) Genethon, Evry, France; (2) Université Paris-Saclay, Univ Evry, Inserm, Généthon, Integrare research unit UMR-S951, Evry, France.

Abstract

We recently identified a signaling pathway that links the upregulation of miR-379 with a mitochondrial response in dystrophic muscle. In the present commentary, we explain the significance that this pathway may have in mitochondrial dysfunction in Duchenne muscular dystrophy (DMD). We identified the upregulation of miR-379 in the serum and muscles of DMD animal models and patients. We found that miR-379 is one of very few miRNAs whose expression was normalized in DMD patients treated with glucocorticoid. We identified EIF4G2 as a miR-379 target, which may promote mitochondrial oxidative phosphorylation (OxPhos) in the skeletal muscle. We found enriched EIF4G2 expression in oxidative fibers, and identified the mitochondrial ATP synthase subunit DAPIT as a translational target of EIF4G2. The identified signaling cascade, which comprises miR-379, EIF4G2 and DAPIT, may link the glucocorticoid treatment in DMD to a recovered mitochondrial ATP synthesis rate. We propose an updated model of mitochondrial dysfunction in DMD.

Key Words: Duchenne Muscular Dystrophy; DLK1-DIO3; miR-379, EIF4G2, Dapit, USMG5, mitochondria; oxidative phosphorylation.

Eur J Transl Myol 31 (3): 10012, 2021 doi: 10.4081/ejtm.2021.10012

Duchenne Muscular Dystrophy (DMD) is an X-linked severe progressive muscle disease caused by mutations in the *DMD* gene, which encodes for the dystrophin protein. The disease affects the motor functions and leads to premature patients death, primarily due to respiratory and cardiac failures.[1] The consequence of the disrupted link between the ECM and the actin cytoskeleton is a process that involves sarcolemma destabilization, perturbation of Ca^{+2} homeostasis, activation of proteases, mitochondrial damage and tissue degeneration.[2]

Of particular interest for this commentary, initial and more recent studies highlighted over the years a critical role for mitochondrial dysfunction in the etiology of DMD.[3] However, the details of the molecular mechanisms of this dysfunction are not yet clear. We recently profiled miRNAs in the plasma and muscles of DMD animal models and patients, and found a large number of dysregulated miRNAs.[4-6] In agreement with other studies, we documented the dysregulation of the myomiRs, which are the skeletal muscle enriched miRNAs, and a few cardiac-muscle-enriched miRNAs. In addition, we identified the dysregulation of a large number of miRNAs which are clustered in the Dlk1-Dio3 genomic locus. The imprinted Dlk1-Dio3 locus hosts the largest miRNA mega-cluster in the human genome, plus other non-coding RNAs and three protein-coding genes (*DLK1*, *RTL1*, and *DIO3*) and is highly conserved in mammalian genomes.[7,8] Dlk1-Dio3 miRNAs (DD-miRNAs) have shown to play a critical role in fetal development and postnatal growth.[9-12] DD-miRNAs are also known to play important roles in embryonic and somatic stem cells.[13-16] Initial indications for Dlk1-Dio3 locus involvement in the muscular system came from the identification of the muscle hypertrophy Callipyge phenotype in the sheep.[17-19] Additionally, patients carrying genetic defects in Dlk1-Dio3 locus present hypotonia and muscle metabolic deficiencies.[20] In the cardiac muscle, DD-miRNAs were shown to regulate diverse functions.[21] In the developing muscle, DD-miRNAs were shown to control the metabolic maturation of muscle precursor cells.[16] However, the biological functions of DD-miRNAs in the context of muscular dystrophy remain relatively unexplored.

Of all dysregulated DD-miRNAs, we decided to focus on miR-379, which was found upregulated in many other muscular dystrophies in addition to DMD.[22] We found that miR-379 is among the most highly expressed and upregulated in DMD plasma. Importantly, it is one of the very few miRNAs whose expression is normalized by glucocorticoid treatment,[6] which is the standard pharmacological care for Duchenne Muscular Dystrophy.[23] We then identified the translation factor *EIF4G2* as a potentially important target and mediator of

miR-379 function in the muscle. *EIF4G2*, a member of the eIF4G translation initiation factors, mediates a cap-independent translation initiation through a mechanism involving the recruitment of the ribosome to specific mRNAs that contain an internal ribosome entry site (IRES) in their 5' untranslated region (UTR).[24] Our attention was drawn to *EIF4G2*, because it was shown recently to promote a mitochondrial shift of glycolytic to OxPhos metabolism, and subsequently of cellular differentiation.[24] Of interest, mitochondrial OxPhos activity was shown to promote myogenic differentiation,[25] suggesting that *EIF4G2* may promote myogenic differentiation by such mechanism. We then noticed that *EIF4G2* is a target gene for miR-139, which similarly to miR-379, belongs to the small group of glucocorticoid-responsive miRNAs in the plasma of DMD patients.[6] Thus, in the dystrophic muscle *EIF4G2* is under a tight regulation, independently by two distinct glucocorticoid-responsive miRNAs, which supported a particular importance of *EIF4G2* in the glucocorticoid response of the dystrophic muscle.

Because *EIF4G2* is a translation factor known to promote the mitochondrial OxPhos, we attempted to identify its putative translation target(s) in the context of mitochondrial activity. In a list of such targets, provided in supplemental information in,[24] we identified among the top hits the mitochondrial protein *DAPIT*, which is encoded by the Usmg5 gene. *DAPIT* struck our attention, because it has been shown previously to be expressed nearly 5-fold higher levels in DMD patients with loss of ambulation at late stage as opposed to early stage,[26] and more recently to be upregulated in the muscle of the neonatal DMD pig model.[27] *DAPIT* is a mitochondrial ATP synthase peripheral stalk subunit, which is required for the dimerization of the ATP Synthase,[28] the shaping of the mitochondrial cristae,[29] and a maximal ATP synthesis rate.[30] Indeed, mutation in Usmg5 gene was found recently in Leigh syndrome patients, characterized by mitochondrial perturbations.[31] We thus hypothesized that *EIF4G2* and *DAPIT* are of interest in DMD as targets of miR-379.

We validated in human myoblasts the targeting of *EIF4G2* by miR-379 and miR-139, and the subsequently downregulation of *DAPIT*, thus experimentally linking the upregulation of miR-379 to reduced *EIF4G2* expression and of *DAPIT* in the myogenic lineage. Immunofluorescence analysis of muscle transversal sections in the mouse confirmed the co-localization of both *EIF4G2* and *DAPIT* to oxidative myofibers. We then knocked down *DAPIT* expression, *in vitro*, in skeletal muscle myotubes, and identified reduced ATP production in the condition of reduced *DAPIT* expression.[32] Finally, treating mice with glucocorticoids increased *EIF4G2* and *DAPIT* expression in skeletal muscle via the reduction of miR-379 level, as seen in DMD patients. Taken together, these findings experimentally confirm a glucocorticoid-responsive signaling pathway in the myogenic lineage that links miR-379 upregulation with a reduced ATP synthesis rate.

In 1975 Mokri end Engel, who investigated muscle biopsies by electron microscopy, identified structural defects in the plasma membrane of DMD myofibers, which permitted the penetration of calcium-rich extracellular fluid.[33,34] Consistently, Wrogemann and Pena, proposed in 1976 the Ca^{+2} hypothesis for the mitochondrial dysfunction in muscular dystrophies.[35] Accordingly, the excessive entry of Ca^{2+} into the damaged myofiber initiates mitochondrial structural with subsequent functional defects reducing ATP production, and promoting a downstream cascade, leading to myofiber degeneration. Forty-five years later, through the discovery in the late eighties of the dystrophin gene and its role in DMD, the basic dogma of mitochondrial dysfunction has not much evolved,[36–38] assuming still that the mitochondria of the dystrophic muscle are merely passively exposed to the external insult of Ca^{+2} overload. Based on,[32] we are suggesting now a modified model for the explanation of mitochondrial dysfunction in DMD, which is presented in figure 1.

The results that were described above support that *EIF4G2* might be involved in the promotion of an oxidative phenotype in the differentiated muscle. As previously mentioned in embryonic stem cells, *EIF4G2* was proposed to modulate cellular differentiation through the promotion of mitochondrial oxidation.[24] Oxidative phosphorylation is also crucial for myogenic differentiation.[25] We identified the enriched expression of *EIF4G2* in oxidative myofibers, in the skeletal muscle.[32] It is possible that, by promoting the translation of *DAPIT* and other mitochondrial OxPhos proteins, *EIF4G2* is an important mediator of the oxidative phenotype in the skeletal muscle. A shift to oxidative phenotype was proposed to be beneficial in DMD,[39] and therefore it is tempting to speculate that overexpression of *EIF4G2* in the muscle may provide protection in DMD.

Another interesting question is concerning the expression pattern and biological functions of *DAPIT*. A number of studies reported *DAPIT*'s mitochondrial localization,[29-31] as a component of the OxPhos complex 5 ATP synthase.[28] In the skeletal muscle of the mouse, high-resolution confocal microscopy shown co-localization of *DAPIT* with *EIF4G2* into oxidative fibers, with however only partial overlap with the mitochondrial ATP synthase ATP5a subunit.[32] Interestingly, in C2C12 myoblast *DAPIT* expression was identified in the lysosome, in addition to the mitochondria, possibly as a component of the lysosomal V-ATPase complex,[40] which is structurally and mechanistically related to the mitochondrial ATP synthase. Thus, *DAPIT* may fulfill a (more than initially anticipated) complex role in the regulation of energy metabolism in the normal and dystrophic skeletal, and cardiac,[41] muscles, which is an interesting challenge for future investigations. Another hypothesis is that DD-miRNAs, other than miR-379, might regulate

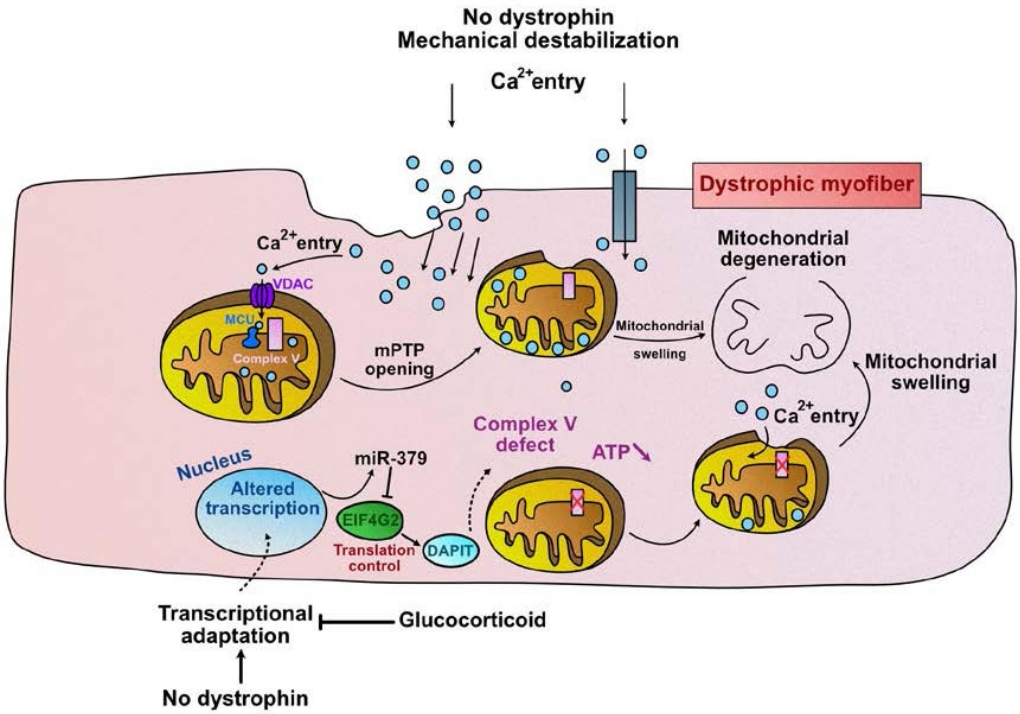

Fig 1. *In the absence of dystrophin, activated miR-379 expression, which subsequently reducing EIF4G2 and DAPIT expression, reducing ATP synthase activity and ATP production, (see main text for details), might synergize with the external Ca^{2+} overload, to result in mitochondrial damage.*

mitochondrial functions in DMD. Clustered miRNAs are thought to coordinately regulate a large number of target transcripts in given signaling pathways.[42,43] Of relevance and as mentioned above, we identified the coordinated upregulation of a large number of DD-miRNAs in the serum and muscles of DMD models and patients. A coordinated simultaneous activity of DD-miRNAs on mitochondrial functions were demonstrated in the hematopoietic system.[14] Moreover, the coordinated targeting of DD-miRNAs of mitochondrial functions was demonstrated in the metabolic regulation of muscle precursor cells.[16] It is therefore tempting to speculate that DD-miRNAs may simultaneously and coordinately regulate mitochondrial functions in myofibers of the regenerating muscle. An ongoing study in our group is toward these directions. In summary, our recent study,[32] exposed a new signaling pathway which is dysregulated in DMD, and contributes to a better understanding of mitochondrial perturbation in DMD.

List of acronyms

DMD - Duchenne Muscular Dystrophy
IRES - Internal Ribosome Entry Site
OxPhos - Oxidative Phosphorylation
UTR – Untranslated Region

Authors' contributions

DI wrote the first draft. MS designed the figure. All authors reviewed, curated and approved the manuscript.

Acknowledgments

We would like to thank Dr. Heather Best for helpful discussions.

Funding

None.

Conflict of Interest

Al authors declare no competing interests.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Corresponding Author

David Israeli, Généthon, 1 rue de l'Internationale, 91000 Evry, France. Tel: +33 (0)1 69 47 29 67
ORCID iD: 0000-0003-2762-2195
E-mail: Israeli@genethon.fr

E-mails and ORCID iDs of Coauthors

Ai Vu Hong: avuhong@genethon.fr
ORCID iD: 0000-0002-0872-4295
Mathilde Sanson: mathildesanson199@gmail.com
ORCID iD: 0000-0002-5184-8891
Isabelle Richard: Richard@genethon.fr
ORCID iD: 0000-0002-6505-446X

References

1. Duan D, Goemans N, Takeda S, Mercuri E, Aartsma-Rus A. Duchenne muscular dystrophy. Nat Rev Dis Prim. 2021;7: 13. 10.1038/s41572-021-00248-3.
2. Allen DG, Whitehead NP, Froehner SC. Absence of Dystrophin Disrupts Skeletal Muscle Signaling: Roles of Ca^{2+} , Reactive Oxygen Species, and Nitric Oxide in the Development of Muscular Dystrophy. Physiol Rev. 2016 Jan;96(1):253-305. doi: 10.1152/physrev.00007.2015.
3. Timpani CA, Hayes A, Rybalka E. Revisiting the dystrophin-ATP connection: How half a century of research still implicates mitochondrial dysfunction in Duchenne Muscular Dystrophy aetiology. Med Hypotheses. 2015 Dec;85(6):1021-33. doi: 10.1016/j.mehy.2015.08.015. Epub 2015 Sep 2.
4. Jeanson-Leh L, Lameth J, Krimi S, Buisset J, Amor F, Le Guiner C, Barthélémy I, Servais L, Blot S, Voit T, Israeli D. Serum profiling identifies novel muscle miRNA and cardiomyopathy-related miRNA biomarkers in Golden Retriever muscular dystrophy dogs and Duchenne muscular dystrophy patients. Am J Pathol. 2014 Nov;184(11):2885-98. doi: 10.1016/j.ajpath.2014.07.021. Epub 2014 Sep 3.
5. Vignier N, Amor F, Fogel P, Duvallet A, Poupiot J, Charrier S, Arock M, Montus M, Nelson I, Richard I, Carrier L, Servais L, Voit T, Bonne G, Israeli D. Distinctive serum miRNA profile in mouse models of striated muscular pathologies. PLoS One. 2013;8(2):e55281. doi: 10.1371/journal.pone.0055281. Epub 2013 Feb 13.
6. Amor F, Vu Hong A, Corre G, Sanson M, Suel L, Blaie S, Servais L, Voit T, Richard I, Israeli D. Cholesterol metabolism is a potential therapeutic target in Duchenne muscular dystrophy. J Cachexia Sarcopenia Muscle. 2021 Jun;12(3):677-693. doi: 10.1002/jcsm.12708. Epub 2021 May 26.
7. da Rocha ST, Edwards CA, Ito M, Ogata T, Ferguson-Smith AC. Genomic imprinting at the mammalian Dlk1-Dio3 domain. Trends Genet. 2008 Jun;24(6):306-16. doi: 10.1016/j.tig.2008.03.011.
8. Tierling S, Dalbert S, Schoppenhorst S, Tsai CE, Oliger S, Ferguson-Smith AC, Paulsen M, Walter J. High-resolution map and imprinting analysis of the Gtl2-Dnchc1 domain on mouse chromosome 12. Genomics. 2006 Feb;87(2):225-35. doi: 10.1016/j.ygeno.2005.09.018. Epub 2005 Nov 23.
9. Chu C, Schwartz S, McPherson E. Paternal uniparental isodisomy for chromosome 14 in a patient with a normal 46,XY karyotype. Am J Med Genet A. 2004 Jun 1;127A(2):167-71. doi: 10.1002/ajmg.a.20618.
10. Ioannides Y, Lokulo-Sodipe K, Mackay DJ, Davies JH, Temple IK. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. J Med Genet. 2014 Aug;51(8):495-501. doi: 10.1136/jmedgenet-2014-102396. Epub 2014 Jun 2.
11. Ogata T, Kagami M. Molecular mechanisms leading to the phenotypic development in paternal and maternal uniparental disomy for chromosome 14. Clin Pediatr Endocrinol. 2008;17(4):103-11. doi: 10.1297/cpe.17.103. Epub 2008 Nov 8.
12 Stevenson DA, Brothman AR, Chen Z, Bayrak-Toydemir P, Longo N. Paternal uniparental disomy of chromosome 14: confirmation of a clinically-recognizable phenotype. Am J Med Genet A. 2004 Sep 15;130A(1):88-91. doi: 10.1002/ajmg.a.30200.
13 Liu L, Luo GZ, Yang W, Zhao X, Zheng Q, Lv Z, Li W, Wu HJ, Wang L, Wang XJ, Zhou Q. Activation of the imprinted Dlk1-Dio3 region correlates with pluripotency levels of mouse stem cells. J Biol Chem. 2010 Jun 18;285(25):19483-90. doi: 10.1074/jbc.M110.131995. Epub 2010 Apr 9.
14. Qian P, He XC, Paulson A, Li Z, Tao F, Perry JM, Guo F, Zhao M, Zhi L, Venkatraman A, Haug JS, Parmely T, Li H, Dobrowsky RT, Ding WX, Kono T, Ferguson-Smith AC, Li L. The Dlk1-Gtl2 Locus Preserves LT-HSC Function by Inhibiting the PI3K-mTOR Pathway to Restrict Mitochondrial Metabolism. Cell Stem Cell. 2016 Feb 4;18(2):214-28. doi: 10.1016/j.stem.2015.11.001. Epub 2015 Nov 25.
15. Stadtfeld M, Apostolou E, Ferrari F, Choi J, Walsh RM, Chen T, Ooi SS, Kim SY, Bestor TH, Shioda T, Park PJ, Hochedlinger K. Ascorbic acid prevents loss of Dlk1-Dio3 imprinting and facilitates generation of all-iPS cell mice from terminally differentiated B cells. Nat Genet. 2012 Mar 4;44(4):398-405, S1-2. doi: 10.1038/ng.1110.
16. Wüst S, Dröse S, Heidler J, Wittig I, Klockner I, Franko A, Bonke E, Günther S, Gärtner U, Boettger T, Braun T. Metabolic Maturation during Muscle Stem Cell Differentiation Is Achieved by miR-1/133a-Mediated Inhibition of the Dlk1-Dio3 Mega Gene Cluster. Cell Metab. 2018 May 1;27(5):1026-1039.e6. doi: 10.1016/j.cmet.2018.02.022. Epub 2018 Apr 5.
17. Gao YQ, Chen X, Wang P, Lu L, Zhao W, Chen C, Chen CP, Tao T, Sun J, Zheng YY, Du J, Li CJ, Gan ZJ, Gao X, Chen HQ, Zhu MS. Regulation of DLK1 by the maternally expressed miR-379/miR-544 cluster may underlie callipyge polar overdominance inheritance. Proc Natl Acad Sci U S A. 2015 Nov 3;112(44):13627-32. doi: 10.1073/pnas.1511448112. Epub 2015 Oct 20.
18. Byrne K, Colgrave ML, Vuocolo T, Pearson R, Bidwell CA, Cockett NE, Lynn DJ, Fleming-Waddell JN, Tellam RL. The imprinted retrotransposon-like gene PEG11 (RTL1) is expressed as a full-length protein in skeletal muscle

from Callipyge sheep. PLoS One. 2010 Jan 8;5(1):e8638. doi: 10.1371/journal.pone.0008638.
19. Fleming-Waddell JN, Olbricht GR, Taxis TM, White JD, Vuocolo T, Craig BA, Tellam RL, Neary MK, Cockett NE, Bidwell CA. Effect of DLK1 and RTL1 but not MEG3 or MEG8 on muscle gene expression in Callipyge lambs. PLoS One. 2009 Oct 9;4(10):e7399. doi: 10.1371/journal.pone.0007399.
20. Kitazawa M, Hayashi S, Imamura M, Takeda S, Oishi Y, Kaneko-Ishino T, Ishino F. Deficiency and overexpression of Rtl1 in the mouse cause distinct muscle abnormalities related to Temple and Kagami-Ogata syndromes. Development. 2020 Sep 2;147(21):dev185918. doi: 10.1242/dev.185918.
21. Dill TL, Naya FJ. A Hearty Dose of Noncoding RNAs: The Imprinted DLK1-DIO3 Locus in Cardiac Development and Disease. J Cardiovasc Dev Dis. 2018 Jul 10;5(3):37. doi: 10.3390/jcdd5030037.
22. Eisenberg I, Eran A, Nishino I, Moggio M, Lamperti C, Amato AA, Lidov HG, Kang PB, North KN, Mitrani-Rosenbaum S, Flanigan KM, Neely LA, Whitney D, Beggs AH, Kohane IS, Kunkel LM. Distinctive patterns of microRNA expression in primary muscular disorders. Proc Natl Acad Sci U S A. 2007 Oct 23;104(43):17016-21. doi: 10.1073/pnas.0708115104. Epub 2007 Oct 17. Erratum in: Proc Natl Acad Sci U S A. 2008 Jan 8;105(1):399.
23. Angelini C, Peterle E. Old and new therapeutic developments in steroid treatment in Duchenne muscular dystrophy. Acta Myol. 2012 May;31(1):9-15.
24. Yoffe Y, David M, Kalaora R, Povodovski L, Friedlander G, Feldmesser E, Ainbinder E, Saada A, Bialik S, Kimchi A. Cap-independent translation by DAP5 controls cell fate decisions in human embryonic stem cells. Genes Dev. 2016 Sep 1;30(17):1991-2004. doi: 10.1101/gad.285239.116.
25. Sin J, Andres AM, Taylor DJ, Weston T, Hiraumi Y, Stotland A, Kim BJ, Huang C, Doran KS, Gottlieb RA. Mitophagy is required for mitochondrial biogenesis and myogenic differentiation of C2C12 myoblasts. Autophagy. 2016;12(2):369-80. doi: 10.1080/15548627.2015.1115172.
26. Pegoraro E, Hoffman EP, Piva L, Gavassini BF, Cagnin S, Ermani M, Bello L, Soraru G, Pacchioni B, Bonifati MD, Lanfranchi G, Angelini C, Kesari A, Lee I, Gordish-Dressman H, Devaney JM, McDonald CM; Cooperative International Neuromuscular Research Group. SPP1 genotype is a determinant of disease severity in Duchenne muscular dystrophy. Neurology. 2011 Jan 18;76(3):219-26. doi: 10.1212/WNL.0b013e318207afeb. Epub 2010 Dec 22.
27. Fröhlich T, Kemter E, Flenkenthaler F, Klymiuk N, Otte KA, Blutke A, Krause S, Walter MC, Wanke R, Wolf E, Arnold GJ. Progressive muscle proteome changes in a clinically relevant pig model of Duchenne muscular dystrophy. Sci Rep. 2016 Sep 16;6:33362. doi: 10.1038/srep33362.
28. He J, Ford HC, Carroll J, Douglas C, Gonzales E, Ding S, Fearnley IM, Walker JE. Assembly of the membrane domain of ATP synthase in human mitochondria. Proc Natl Acad Sci U S A. 2018 Mar 20;115(12):2988-2993. doi: 10.1073/pnas.1722086115. Epub 2018 Feb 12.
29. Siegmund SE, Grassucci R, Carter SD, Barca E, Farino ZJ, Juanola-Falgarona M, Zhang P, Tanji K, Hirano M, Schon EA, Frank J, Freyberg Z. Three-Dimensional Analysis of Mitochondrial Crista Ultrastructure in a Patient with Leigh Syndrome by In Situ Cryoelectron Tomography. iScience. 2018 Aug 31;6:83-91. doi: 10.1016/j.isci.2018.07.014. Epub 2018 Jul 20.
30. Ohsakaya S, Fujikawa M, Hisabori T, Yoshida M. Knockdown of DAPIT (diabetes-associated protein in insulin-sensitive tissue) results in loss of ATP synthase in mitochondria. 2011;286.
31. Barca E, Ganetzky RD, Potluri P, Juanola-Falgarona M, Gai X, Li D, Jalas C, Hirsch Y, Emmanuele V, Tadesse S, Ziosi M, Akman HO, Chung WK, Tanji K, McCormick EM, Place E, Consugar M, Pierce EA, Hakonarson H, Wallace DC, Hirano M, Falk MJ. USMG5 Ashkenazi Jewish founder mutation impairs mitochondrial complex V dimerization and ATP synthesis. Hum Mol Genet. 2018 Oct 1;27(19):3305-3312. doi: 10.1093/hmg/ddy231.
32. Sanson M, Vu Hong A, Massourides E, Bourg N, Suel L, Amor F, Corre G, Bénit P, Barthélémy I, Blot S, Bigot A, Pinset C, Rustin P, Servais L, Voit T, Richard I, Israeli D. miR-379 links glucocorticoid treatment with mitochondrial response in Duchenne muscular dystrophy. Sci Rep. 2020 Jun 4;10(1):9139. doi: 10.1038/s41598-020-66016-7.
33. Mokri B, Engel AG. Duchenne dystrophy: electron microscopic findings pointing to a basic or early abnormality in the plasma membrane of the muscle fiber. Neurology. 1975 Dec;25(12):1111-20. doi: 10.1212/wnl.25.12.1111.
34. Mokri B, Engel AG. Commentary. Neurology. 1998;51:1–1.
35. Wrogemann K, Pena SD. Mitochondrial calcium overload: A general mechanism for cell-necrosis in muscle diseases. Lancet. 1976 Mar 27;1(7961):672-4. doi: 10.1016/s0140-6736(76)92781-1.
36. Zulian A, Schiavone M, Giorgio V, Bernardi P. Forty years later: Mitochondria as therapeutic targets in muscle diseases. Pharmacol Res. 2016 Nov;113(Pt A):563-573. doi: 10.1016/j.phrs.2016.09.043. Epub 2016 Sep 30.
37. Law ML, Cohen H, Martin AA, Angulski ABB, Metzger JM. Dysregulation of Calcium Handling in

Duchenne Muscular Dystrophy-Associated Dilated Cardiomyopathy: Mechanisms and Experimental Therapeutic Strategies. J Clin Med. 2020 Feb 14;9(2):520. doi: 10.3390/jcm9020520.

38. Mareedu S, Million ED, Duan D, Babu GJ. Abnormal Calcium Handling in Duchenne Muscular Dystrophy: Mechanisms and Potential Therapies. Front Physiol. 2021 Apr 9;12:647010. doi: 10.3389/fphys.2021.647010.
39. Ljubicic V, Burt M, Jasmin BJ. The therapeutic potential of skeletal muscle plasticity in Duchenne muscular dystrophy: phenotypic modifiers as pharmacologic targets. FASEB J. 2014 Feb;28(2):548-68. doi: 10.1096/fj.13-238071. Epub 2013 Nov 18.
40. Kontro H, Hulmi JJ, Rahkila P, Kainulainen H. Cellular and tissue expression of DAPIT, a phylogenetically conserved peptide. Eur J Histochem. 2012 May 22;56(2):e18. doi: 10.4081/ejh.2012.18
41. Nagata Y, Yamagishi M, Konno T, Nakanishi C, Asano Y, Ito S, Nakajima Y, Seguchi O, Fujino N, Kawashiri MA, Takashima S, Kitakaze M, Hayashi K. Heat Failure Phenotypes Induced by Knockdown of DAPIT in Zebrafish: A New Insight into Mechanism of Dilated Cardiomyopathy. Sci Rep. 2017 Dec 12;7(1):17417. doi: 10.1038/s41598-017-17572-y. Erratum in: Sci Rep. 2018 May 14;8(1):7768.
42. Wang Y, Luo J, Zhang H, Lu J. microRNAs in the Same Clusters Evolve to Coordinately Regulate Functionally Related Genes. Mol Biol Evol. 2016 Sep;33(9):2232-47. doi: 10.1093/molbev/msw089. Epub 2016 Apr 28.
43. Cantini L, Bertoli G, Cava C, Dubois T, Zinovyev A, Caselle M, Castiglioni I, Barillot E, Martignetti L. Identification of microRNA clusters cooperatively acting on epithelial to mesenchymal transition in triple negative breast cancer. Nucleic Acids Res. 2019 Mar 18;47(5):2205-2215. doi: 10.1093/nar/gkz016.

Submitted: August 3, 2021
Revision received: August 31, 2021
Accepted for publication: September 12, 2021