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ORIGINAL ARTICLE

Purkinje cell loss associated with specific ultrastructural alterations in a mouse model with transgenic expression of mutated ataxin-2

Pérdida de células de Purkinje asociada con alteraciones ultraestructurales específicas en un modelo de ratón con expresión transgénica de ataxina-2 mutada

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ABSTRACT

Introduction: The expression of ataxin-2 protein with an N-terminal expanded polyglutamine domain is the cause of Spinocerebellar Ataxia type 2 (SCA2), acting primarily on cerebellar Purkinje cells. The morphological study of cerebellar Purkinje cells expressing polyglutamine-expanded ataxin-2 might provide information on the pathogenic mechanisms underlying the disease process and contribute to the identification of potential therapeutic targets.

Objective: To identify changes in the cellularity and ultrastructure of Purkinje cells associated with the expression of polyglutamine-expanded ataxin-2.

Material and Methods: Three groups of transgenic mice (F066) aged two, five, and 12 months, expressing the polyglutamine-expanded ataxin-2 and a group of 12-month-old wild type mice, were studied. Histological slides of the Purkinje cell layer were prepared from each group for conventional and electron microscopic analysis.

Results: Consistent with previous validation studies, a significant reduction ($p<0.05$) of Purkinje cell density was observed in F066 mice at five (14.82±2.61) and 12 (13.9±0.58) months of age relative to the control group (23.77±0.46). Similarly, ultrastructural alterations consisting of the loss of mitochondrial cristae, dilation of the rough endoplasmic reticulum and Golgi complex were observed in all three groups of transgenic animals.

Conclusions: Transgenic expression of polyQ-expanded ataxin-2 in the F066 model is associated with progressive degeneration and Purkinje cells ultrastructural damage. Consequently, these results suggest that polyglutane-expanded ataxin-2 could exert its neurotoxic effect through functional alterations of the endoplasmic reticulum, Golgi complex, and mitochondria.

Keywords:
Ataxia, Spinocerebellar Ataxia type 2, ataxin-2, transgenic mice, polyglutamine disorders, Purkinje cells.

RESUMEN

Introducción: La expresión de la proteína ataxina-2 con un dominio poliglutamínico expandido en su extremo N-terminal es la causa de la Ataxia Espinocerebelosa tipo-2, afectando primariamente a las células de Purkinje cerebelosas. El estudio morfológico de esta población neuronal expresando la ataxina-2 mutada podría brindar información sobre los mecanismos patogénicos subyacentes al proceso de enfermedad y contribuir a la identificación de potenciales dianas terapéuticas.

Objetivo: Identificar cambios en la celularidad y ultraestructura de células de Purkinje asociados a la expresión de la ataxina-2 con expansiones poliglutamínicas.

Material y Métodos: Tres grupos de ratones transgénicos (F066) de dos meses, cinco meses y 12 meses de edad expresando ataxina-2 con expansiones poliglutamínicas y un grupo de ratones salvajes de 12 meses de edad, fueron estudiados. Láminas histológicas de la capa de células de Purkinje correspondientes a cada grupo de animales fueron analizadas por microscopía convencional y electrónica.

Resultados: Consistente con estudios previos, una significativa reducción ($p<0,05$) en la densidad de células de Purkinje fue observada en los ratones F066 a los cinco (14,82±2,61) y 12 (13,9±0,58) meses de edad comparado con el grupo control (23,77±0,46). De manera similar, alteraciones ultraestructurales consistentes en pérdida de crestas mitocondriales, dilatación del retículo endoplasmático y del complejo de Golgi, fueron observadas en los tres grupos de animales transgénicos.

Conclusiones: La expresión transgénica de la ataxina-2 con expansiones poliglutamínicas está asociada con degeneración progresiva y con daños ultraestructurales de las células de Purkinje en el modelo F066. En consecuencia, los resultados sugieren que la ataxina-2 con expansiones poliglutamínicas podría ejercer su efecto neurotóxico a través de alteraciones funcionales del retículo endoplasmático, complejo de Golgi y mitocondrias.

Palabras Claves:
Ataxia, Ataxia Espinocerebelosa tipo-2, ataxina-2, ratones transgénicos, desórdenes poliglutamínicos, células de Purkinje.

INTRODUCTION

Spinocerebellar ataxia type 2 (SCA2) belongs to a group of hereditary neurodegenerative diseases caused by expansion of a CAG repeat tract in coding regions of novel genes. Collectively referred as polyglutamine (polyQ) disorders, so far this group of diseases also include Spinal and Bulbar Muscular atrophy, Huntington's disease, Dentatorubral-Pallidoluysian atrophy, SCA1, SCA3 (Machado-Joseph disease), SCA6, SCA7, and SCA17.[1,2,3] In particular, SCA2 is due to the expansion by 32 or more CAG repeats in the *ATXN2* gene (locus 12q24.1), encoding for the ataxin-2 protein with an expanded polyglutamine domain in the N-terminal region.[4,5] PolyQ-expanded ataxin-2 leads to conspicuous atrophy of cerebellar Purkinje cells, thalamic and cholinergic basal forebrain neurons, brain stem pontine and olivary neurons, and spinal and cortical motor neurons,[6] probably because of the combined action of both gain and loss of function mechanisms resulting from the mutation.[7] This neuropathological pattern is translated clinically into a cerebellar syndrome with progressive gait ataxia, cerebellar dysarthria, dysmetria, dysdiadochokinesia, leg cramps, kinetic or postural tremor, decreased tendon reflexes, abnormal eye movements, and decreased survival.[8,9]

Several studies have shown that ataxin-2 is essentially a cytoplasmic RNA binding protein that has been associated with cellular stress responses via the mechanistic target of rapamycin complex 1 (mTORC1) signaling and mRNA translation modulation.[7,10] Besides, ataxin-2 has been involved in lipid storage and energy metabolism,[11,12,13] and in the modulation of circadian rhythms.[14,15] Also, ataxin-2 seems to be important for proper mitochondrial function.[16,17] Normally, ataxin-2 is localized at the rough endoplasmic reticulum,[9,18] at the Golgi complex[19] or sites of receptor tyrosine kinases endocytosis in the plasma membrane.[20,21,22,23]

The pathologic expansion of the polyQ domain in ataxin-2 has been associated with structural disruption of subcellular components and the activation of apoptotic cell death pathways.[19,23] In particular, the expression of polyQ-expanded ataxin-2 disrupted the normal morphology of the Golgi complex in transiently transfected COS1 cells[19] and C. elegans embryos.[24] Besides, strong evidence has been obtained for ataxin-2 regulating proper ER morphology and dynamics.[18,24] However, no ultrastructural studies have been performed to further characterize morphological alterations in subcellular compartments potentially involved in the neurodegenerative process resulting from polyQ-expanded ataxin-2 expression.

Since human SCA2 autopsy material is very scarce, this study was carried out in the F066 SCA2 transgenic mouse model[25] with the aim of exploring by morphometric and electron microscopy methods the potential effects of polyQ-expanded ataxin-2 protein expression on the cellularity and ultrastructure of cerebellar Purkinje cells. Overall, our study confirmed the progressive loss of Purkinje cells in the F066 model and defines ultrastructural changes in the endoplasmic reticulum (ER), Golgi complex (GC), and mitochondria of neurons still present. Finally, it is suggested that polyQ-expanded ataxin-2 exerts its neurotoxic effects through the structural disruption and subsequent dysfunction of these subcellular compartments and a contribution to the validation of the F066 model as an experimental tool for SCA2 is made.

The **objective** of this research is to identify changes in the cellularity and ultrastructure of Purkinje cells associated with the expression of polyglutamine-expanded ataxin-2.

MATERIALS AND METHODS

This study corresponds to a case-control design. It was carried out at the Center for Genetic Engineering and Biotechnology in Havana, Cuba, between the months of October 2004 and December 2006.

Animals

Twenty male mice were studied, including 15 transgenic homozygous mice (F066) carrying a human *ATXN2* transgene with 75 CAG repeats, and five non-transgenic/wild type mice, all in a B6D2F1 X OF1 background. The animals were maintained on a 12/12-hour dark/light cycle (lights were on from 8 a.m. to 8 p.m.), with free access to food and water. The manipulation was performed following the ethical Principles for the use and care of Laboratory Animal of the Center for Genetic Engineering and Biotechnology (CIGB). A maximum of two males by litter was used to avoid any "litter effect". Animals with signs of respiratory distress or tumors were excluded. Four groups of five animals each were assembled: two-month-old transgenic mice (TG1), five-month-old transgenic mice (TG2), 12 month-old transgenic mice (TG3), and 12 month-old non-transgenic mice used as the control group (C). Four mice from each group were sacrificed by cervical dislocation and their brains were immediately removed and processed for morphometric analysis. On the other hand, the remaining animal in each group was sacrificed with pentobarbital and transcardially perfused with 3% glutaraldehyde in 0.1 M cacodylate buffer. Subsequently, the cerebellum was removed and processed for electron microscopy analysis.

Morphometric analysis

Brains were fixed in 10% formalin and paraffin-embedded. Two sagittal sections of the left cerebellar hemispheres (0.12 and 1.20 according to Paxinos stereotaxic mouse atlas) of each animal were dyed with cresyl violet. Digital images of the visual fields were taken throughout the cerebellar cortex at 40X magnification using a trinocular optical microscope (Zeiss AxioSkop 40). The digital images were stored on a PC and processed for morphometric analysis using the NIH ImageJ software package (previously calibrated with a micrometric ruler). The number of Purkinje cells was counted and the length of the intermediate layer of the cerebellar cortex in each field was measured (1000 µm=1mm). Purkinje cell density was calculated by dividing the cell number by the length of the intermediate layer. The results were expressed as cellular density/mm of length.

Electron microscopy analysis

Cerebellar cortex samples were taken from the remaining animal in each group at the same level as for morphometric analyses. These were post-fixed with 1% osmium tetraoxide, dehydrated in a series of ethanol and embedded in Epon 812.[26] Samples were cut into ultrafine sections of 40-50 nm and placed on copper grids (two by animal), which were stained with uranyl acetate followed by lead citrate and examined in a transmission electron microscopy JEM 2000 (Jeol, Japan). The Purkinje cells analyses were performed by an investigator that was blind to the study groups. By random sampling, a total of twenty cerebellar Purkinje cells were evaluated on two grids by an animal. Finally, the percentage of cells with morphological abnormalities was recorded as a measure of damage for each group.

Statistical analysis

GraphPad InStat-prism (version 6.0) statistical package was used for statistical data processing. The Kruskal-Wallis test was used to determine whether there were significant differences between comparison groups regarding Purkinje cell density. The Dunn's multiple comparison test was used to determine between which comparison groups there were significant differences for Purkinje cell density.

RESULTS

Purkinje cell density

Contrary to other neuronal types, Purkinje cells are arranged linearly side by side throughout the intermediate layer of the cerebellar cortex and, given the morphology of this region, they occupy different planes.[27,28] This means that there is a great topological diversity among microscopic fields and therefore these cannot be used as a common denominator to evaluate the density of this neuronal type. Determining the length of the interlayer linearly in each field removes this limitation. In the present study, considering the three groups of transgenic animals and the two histological sections of each animal, 717 microscopic fields were recorded, which totaled 5768 Purkinje cells and 368.6 mm in length (per group [number of fields/number of cells/length]: TG1: 226/2139/118.19; TG2: 255/1937/129.41; TG3: 236/1692/121). On the other hand, a total of 232 microscopic fields and 2892 cells extending 121.24 mm in length were recorded in the control group.

The mean (SD) for Purkinje cell densities in study groups were as follows: C: 23.77 (0.46); TG1: 17.49 (1.09); TG2: 14.82 (2.61) and TG3: 13.9 (0.58). Highly significant differences were obtained between comparison groups (K-W=11.40; p=0.0009). In particular, TG2 and TG3 showed a significant reduction in Purkinje cell density relative to the control group. Though there were no significant differences for Purkinje cell density between TG groups, it was evidenced a trend towards a greater reduction with advancing age (Figure 1).

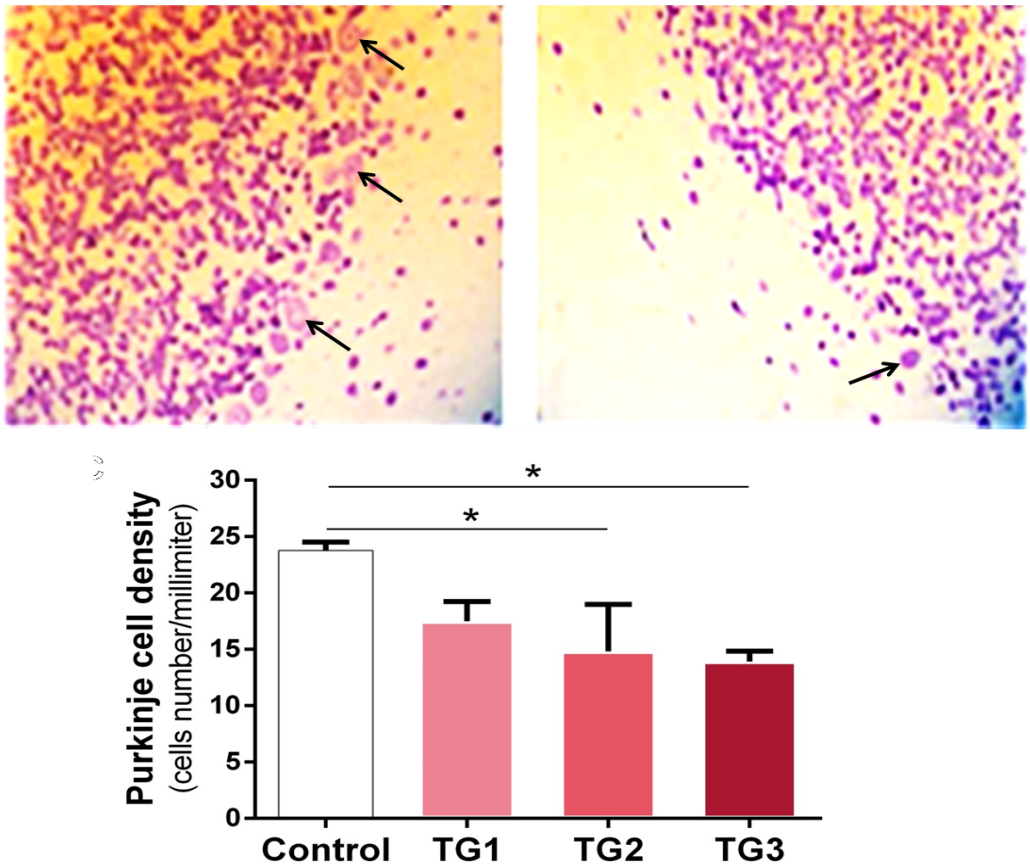

Figure 1. Qualitative and quantitative analysis of the cerebellar Purkinje cell layer in the F066 mouse model. A- Cresyl violet staining of cerebellar Purkinje cells of nontransgenic mice with twelve-month-old showing abundant Purkinje cells (indicated by black arrows); B- Cresyl violet staining of cerebellar Purkinje cells of two-month-old transgenic mice showing a conspicuous loss of Purkinje cells (indicated by black arrows). C- Quantitative analysis of Purkinje cell density by comparison groups. Control: twelve-month-old non-transgenic mice group; TG1: Two-month-old transgenic mice group; TG2: five-month-old transgenic mice group; TG3: twelve-month-old transgenic mice group.

Ultrastructural changes in subcellular compartments

No ultrastructural alterations were evident in the control group at both nuclear and cytoplasmic compartments (Figure 2A). In contrast, 40% (8/20) of cells in TG1, 60% (12/20) in TG2, and 50% (10/20) in TG3 showed subcellular alterations consisting of ER (Figure 2B-D) and GC dilation (Figure 2C and D), as well as the loss of the mitochondrial cristae (Figure 2B and C).

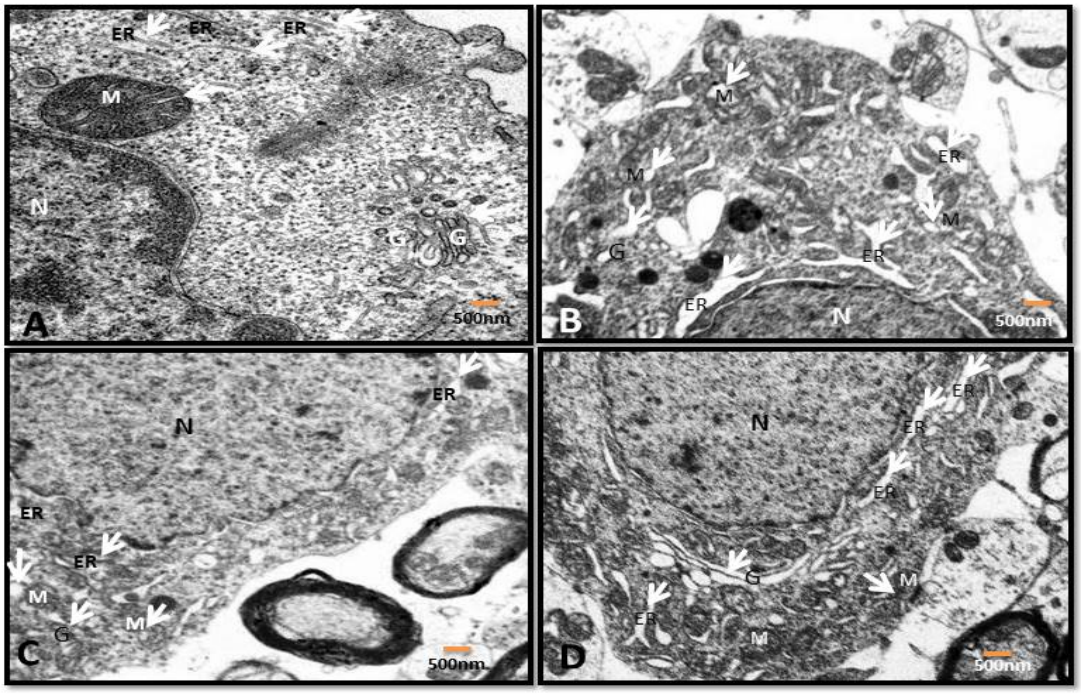

Figure 2. Electron micrographs representative of cerebellar Purkinje cells from transgenic and non-transgenic mice. A- 12-month-old non-transgenic mice group (Magnification 12000); B- two-month-old transgenic mice group (Magnification 12000); C- five-month-old transgenic mice group (Magnification 12000); D- 12-month-old transgenic mice group (Magnification 12000); The white arrows point to the location of subcellular structures. M: mitochondria; ER: endoplasmic reticulum; G: Golgi complex; N: nucleus. Barr=500nm.

DISCUSSION

Since the discovery of the causal SCA2 mutation in 1996, significant progress has been made in understanding its biological basis. Even so, none of the results obtained so far translated in effective therapeutic options, which has devastating consequences for affected individuals and forces us to continue delving in the characterization of the diseases process.

In 2006, the publication of this work was held back by the interest in replicating it through a broader design, which was ultimately not possible. Since then, a significant number of similar studies have been carried out, which taking into account the characteristics of the F066 experimental model and current design, support the validity and novelty of our observations and therefore, the importance of their publication, even almost two decades later.

The generation and characterization of F066 mice has been previously described by Aguiar *et al*.[25] Corresponding to the ubiquitous expression of the *ATXN2* gene in humans, expression of the *ATXN2* transgene with 75 CAG repeats was demonstrated at different levels and structures, including at the protein level in the cerebellum. Furthermore, as in SCA2-affected individuals, alterations of motor functions and an evident decrease in the number of Purkinje cells were described in the F066 model.[25]

Taken together, the above results pointed to the F066 model as a useful experimental model for the study of SCA2. However, the qualitative nature of the method of assessing Purkinje cellularity and the fact that the effects of time of exposure to the mutated protein were not considered, limit the practical usefulness of this analysis.[25] In contrast, our design, based on the evaluation of Purkinje cell density, by showing a significant reduction of this neuronal population from five months of age, corroborates the effect on this neuronal population caused by the expression of mutated ataxin-2 in humans and contributes to the validation of the F066 model as an experimental system for SCA2. Additionally, it allows us to suggest that any attempt to evaluate potential therapeutic options in the model with certainty, must contemplate ages less than five months. However, future studies that consider equivalent age groups between transgenic and control ones would be necessary since our design does not allow us to objectively assess the effect of mutated ataxin-2 on the density of Purkinje cells in 2-month-old transgenic mice.

On the other hand, electron microscopy analysis provides information concerning the integrity of the cellular ultrastructure and in degenerating cells could guide on the involvement of specific subcellular structures and consequently on potential underlying pathogenic mechanisms.

Contrary to other polyglutamine diseases, where the pathogenic process has been linked with misfolding and intranuclear aggregation of mutated proteins[24,29] in SCA2, the cytoplasmic localization of ataxin-2 and aggregate corresponding in degenerating Purkinje cells suggest that intranuclear localization is not necessary for the pathogenic process.[29] Our ultrastructural analysis is consistent with these observations and with previous studies relating the ataxin-2 protein with the morphology and dynamics of the endoplasmic reticulum, Golgi complex, and mitochondria.

Huynh *et al*.,[19] detected normal ataxin-2 in the cell fraction corresponding to CG in the human cerebral cortex and transfected COS-1 cells. Also, they observed that expression of the mutated variant in COS-1 cells is associated with GC disruption and increased markers of apoptotic cell death.[19] Similar results regarding the localization of ataxin-2 in the GC were also described by Turnbull *et al*.[30] However, in both studies the specificity of the antibodies used for ataxin-2 immunolocalization was not tested in Sca2 knock-out tissue. In addition, due to the use by Huynh et al., of brefeldin-A.,[19] an agent that causes colocalization of specific GC and ER markers, the potential localization of ataxin-2 also in ER cannot be ruled out. Correspondingly, a subsequent study by S. van de Loo *et al*.,[18] also in cellular models, describes the localization of ataxin-2 both normal and with expanded repeats preferably in the ER.[18] The link between ataxin-2 and ER was also observed in studies conducted by Castillo *et al*.[24] In this case, consistent with a partial loss-of-function mechanism, the depletion of proteins homologous to human ataxin-2 in *C. elegans* and *Drosophila melanogaster* (ATX-2 and Atx2, respectively), resulted in the collapse of the ER in embryonic cells and germline. The ER morphology and dynamics were also severely affected in neuronal cultures of drosophila in this study, with the recapitulation of shrunken dendritic arbor phenotype of SCA2. The authors also described structural compromises of the mitochondria, which appear longer and more elongated.[24] An altered mitochondrial morphology was also described by Cornelius et al., on cultured fibroblasts from SCA2 patients.[31] Taken together with our results, such observations suggest that the loss of Purkinje cells evidenced in the F066 model could result from the pathogenic effect of mutated ataxin-2 on the structure and function of the ER, GC, and mitochondria.

In general, structural disruption of the ER, GC, and mitochondria has been linked to the pathogenesis of neurodegenerative diseases in different experimental contexts.[31,32,33] The dilation or widening of the ER tubules has been described as a survival mechanism that allows more time to correct misfolded proteins, as well as to increase their capacity to sequester large amounts of Ca2+, thus avoiding compromises of cellular homeostasis.[34] From this perspective, a potential mechanism linking mutated ataxin-2 to neuronal degeneration would be one where the mutation leads to its misfolding, aggregation, and accumulation in the ER causing ER stress and apoptosis. On the other hand, the Golgi complex is a cytoplasmic organelle whose structural organization is essential for the sorting and modification of proteins synthesized by the ER.[33] It has been hypothesized that it undergoes structural disruption under stress conditions and that this acts as a mechanism inducing stress response. When it is ineffective, the affected cells may undergo apoptosis.[35] In addition to ER and GC, mitochondria also play important roles in cellular functioning, especially in neurons due to their higher energy demand.[36,37,38] It has been described that remodeling of the mitochondrial cristae occurs during apoptosis to allow the release of apoptotic factors.[39]

In another direction, given that ultrastructural disruptions were evidenced from 2 months of age, future studies that contemplate lower ages are needed. The Ataxin-2 protein is expressed ubiquitously and very early in the developmental process,[40] and massive CAG repeat expansions in the *ATXN2* gene have been associated with congenital SCA2 presentations.[41] Besides, the transgene used in the F066 model carries the largest expansion recorded in the Cuban SCA2 population,[25] corresponding to a patient who was affected by the age of four years.

Finally, this research is one of the few studies ever conducted on electron microscopy analysis of Purkinje cells expressing a poly-Q expanded Ataxin-2. Previous studies conducted in a 58Q Ataxin-2 transgenic mouse model did not assess the potential damage to the cellular substructure,[42,43] such as those described here. Other analyzes were carried out by biochemical or morphological methods that, due to their scope do not allow an objective assessment of the potential effect of the mutated protein on subcellular structure or focused on experimental systems phylogenetically more distant from humans.

CONCLUSIONS

In conclusion, in the present study it was observed that transgenic expression of poly-Q expanded Ataxin-2 is associated with a progressive loss of cerebellar Purkinje cells and with ultrastructural disruptions involving the ER, GC and mitochondria. Consequently, although future studies are needed, these results suggest that poly-Q expanded Ataxin-2 could exert its pathogenic effect through functional alterations of these organelles. Finally, the results contributed to validate the usefulness of the F066 transgenic mice as an experimental model for SCA2.

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REFERENCES

1. Paulson HL, Shakkottai VG, Clark HB. Polyglutamine spinocerebellar ataxias - from genes to potential treatments. Nat. Rev. Neurosci [Internet]. 2017;18(10):613–626. Available from: https://doi.org/10.1038/nrn.2017.92

2. Ashizawa T, Öz G, Paulson HL. Spinocerebellar ataxias: prospects and challenges for therapy development. Nat Rev Neurol [Internet]. 2018;14(10):590-605. Available from: https://doi.org/10.1038/s41582-018-0051-6

3. Schultz JL, Moser AM, Nopoulos PC. The Association between CAG Repeat Length and Age of Onset of Juvenile-Onset Huntington's Disease. Brain Sci [Internet]. 2020;10(9):575. Available from: https://doi.org/10.3390/brainsci10090575

4. Satterfield TF, Jackson SM, Pallanck LJ. A Drosophila Homolog of the Polyglutamine Disease Gene SCA2 Is a Dosage-Sensitive Regulator of Actin Filament Formation. Genetics [Internet]. 2002;162(4):1687-702. Available from: https://doi.org/10.1093/genetics/162.4.1687

5. Pang JT, Giunti P, Chamberlain S. Neuronal intranuclear inclusions in SCA2: a genetic, morphological and immunohistochemical study of two case. Brain [Internet]. 2002;125(Pt3):656-63. Available from: https://doi.org/10.1093/brain/awf060

6. Rüb U, Schöls L, Paulson HL. Clinical features, neurogenetics and neuropathology of the poly-glutamine spinocerebellar ataxias type 1, 2, 3, 6 and 7. Prog Neurobiol [Internet]. 2013;104:38-66. Available from: https://doi.org/10.1016/j.pneurobio.2013.01.001

7. Lastres IB, Nonis D, Eich F. Mammalian ataxin-2 modulates translation control at the pre-initiation complex via PI3K/mTOR and is induced by starvation. Biochim Biophys Acta [Internet]. 2016;1862(9):1558-69. Available from: https://doi.org/10.1016/j.bbadis.2016.05.017

8. Auburger GW. Spinocerebellar ataxia type 2. Handb Clin Neurol [Internet]. 2012;103:423-36. Available from: https://doi.org/10.1016/B978-0-444-51892-7.00026-7

9. Almaguer ML., Aguilera RR, González ZY. Estimation of survival in Spinocerebellar ataxia type 2 Cuban patients. Clin Genet [Internet]. 2013;83(3):293-294. Available from: https://doi.org/10.1111/j.1399-0004.2012.01902.x

10. Fittschen M, Lastres IB, Halbach MV. Genetic ablation of ataxin-2 increases several global translation factors in their transcript abundance but decreases translation rate. Neurogenet [Internet]. 2015;16(3):181-92. Available from: https://doi.org/10.1007/s10048-015-0441-5

11. Damrath E, Heck MV, Gispert S. ATXN2-CAG42 sequesters PABPC1 into insolubility and induces FBXW8 in cerebellum of old ataxic knock-in mice. PLoS Genet [Internet]. 2012;8(8):e1002920. Available from: https://doi.org/10.1371/journal.pgen.1002920

12. Dansithong W, Paul S, Figueroa KP. Ataxin-2 regulates RGS8 translation in a new BAC-SCA2 transgenic mouse model. PLoS Genet [Internet]. 2015;11(14):e1005182. Available from: https://doi.org/10.1371/journal.pgen.1005182

13. Sen NE, Canet PJ, Halbach MV. Generation of an Atxn2-CAG100 knock-in mouse reveals N-acetylaspartate production deficit due to early Nat8l dysregulation. Neurobiol Dis [Internet]. 2019;132:104559. Available from: https://doi.org/10.1016/j.nbd.2019.104559

14. Lim C, Allada R. ATAXIN-2 activates PERIOD translation to sustain circadian rhythms in drosophila. Science [Internet]. 2013;340(6134):875-9. Available from: https://doi.org/10.1126/science.1234785

15. Zhang Y, Ling J, Yuan C. A role for drosophila ATX2 in activation of PER translation and circadian behavior. Science [Internet]. 2013;340(6134):879-82. Available from: https://doi.org/10.1126/science.1234746

16. Meierhofer D, Halbach M, Sen NE. Ataxin-2 (Atxn2)-Knock-out mice show branched chain amino acids and fatty acids pathway alterations. Mol Cell Proteomics [Internet]. 2016;15(5):1728-39. Available from: https://doi.org/10.1074/mcp.M115.056770

17. Yang YS, Kato M, Wu X. Yeast Ataxin-2 Forms an Intracellular Condensate Required for the Inhibition of TORC1 Signaling during Respiratory Growth. Cell [Internet]. 2019;177(3):697-710.e17. Available from: https://doi.org/10.1016/j.cell.2019.02.043

18. Van de Loo S, Eich F, Nonis D. Ataxin-2 associates with rough endoplasmic reticulum. Exp Neurol [Internet]. 2009;215(1):110-8. Available from: https://doi.org/10.1016/j.expneurol.2008.09.020

19. Huynh DP, Yang HP, Vakharia H. Expansion of the polyQ repeat in ataxia-2 alters its Golgi localization, disrupts the Golgi complex and causes cell death. Human Molecular Genetics [Internet]. 2003;12(13):1485-96. Available from: https://doi.org/10.1093/hmg/ddg175

20. Drost J, Nonis D, Eich F. Ataxin-2 modulates the levels of Grb2 and SRC but not ras signaling. Journal of Molecular Neuroscience [Internet]. 2013;51(1):68-81. Available from: https://doi.org/10.1007/s12031-012-9949-4

21. Nonis D, Schmidt MH, van de Loo S. Ataxin-2 associates with the endocytosis complex and affects EGF receptor trafficking. Cellular Signalling [Internet]. 2008;20(10):1725-39. Available from: https://doi.org/10.1016/j.cellsig.2008.05.018

22. Ralser M, Nonhoff U, Albrecht M. Ataxin-2 and huntingtin interact with endophilin-A complexes to function in plastin-associated pathways. Human Molecular Genetics [Internet]. 2005;14(19):2893-909. Available from: https://doi.org/10.1093/hmg/ddi321

23. Canet PJ, Nesli ES, Aleksandar A. Atxn2-CAG100-KnockIn mouse spinal cord shows progressive TDP43 pathology associated with cholesterol biosynthesis suppression. Neurobiol Dis [Internet]. 2021;152:105289. Aviable from: https://doi.org/10.1016/j.nbd.2021.105289

24. Castillo U, Gnazzo MM, Turpin CG. Conserved role for Ataxin-2 in mediating endoplasmic reticulum dynamics. Traffic [Internet]. 2019;20(6):436-47. Available from: https://doi.org/10.1111/tra.12647

25. Aguiar J, Fernández J, Aguilar A. Ubiquitous expression of human SCA2 gene Ander the regulation of the SCA2 self-promoter cause specific Purkinje cell degeneration in transgenic mice. Neuroscience Letters [Internet]. 2006;392(3):202-6. Available from: https://doi.org/10.1016/j.neulet.2005.09.020

26. Spurr AR. A low-viscosity epoxy resin embed-ding medium for electron microscopy. J. Ultra-struct. Res [Internet]. 1969;26(1):31-43. Available from: https:// doi.org/10.1016/S0022-5320(69)90033-1

27. Sereno MI, Diedrichsen J, Tachrount M, et al. The human cerebellum has almost 80% of the surface area of the neocortex. Proc Natl Acad Sci U S A [Internet]. 2020;117(32):19538-19543. Available from: https://doi.org/10.1073/pnas.2002896117

28. Ilhami C, Muzaffer S, Ahmet S. Histological and histomorphometric studies on the cerebellar cortex and silver stained nucleolus organizer regions of Purkinje neurons in chronic morphine-treated rats. Veterinarski Arhiv [Internet]. 2018;8(1):75-88. Available from: https://doi.org/10.24099/vet.arhiv.160902ª

29. Huynh DP, Figueroa K, Hoang N. Nuclear localization or inclusion body formation of ataxin-2 is not necessary for SCA2 pathogenesis in mouse or human. Nat Genet [Internet]. 2000;26(1):44-50. Available from: https://doi.org/10.1038/79162

30. Turnbull VJ, Storey E, Tarlac V. Different ataxin-2 antibodies display different immunoreactive profiles. Brain Res [Internet]. 2004;1027(1-2):103–16. Available from: https://doi.org/10.1016/j.brainres.2004.08.044

31. Cornelius N, Wardman JH, Hargreaves IP. Evidence of oxidative stress and mitochondrial dysfunction in spinocerebellar ataxia type 2 (SCA2) patient fibroblasts: Effect of coenzyme Q10 supplementation on these parameters. Mitochondrion [Internet]. 2017;34:103-14. Available from: https://doi.org/10.1016/j.mito.2017.03.001

32. Cabral FM, Hetz C. ER Stress and Neurodegenerative Disease: A Cause or Effect Relationship?. Curr Top Microbiol Immunol [Internet]. 2018;414:131-157. Available from: https://doi.org/10.1007/82_2017_52

33. Nakagomi S, Barsoum MJ, Wetzel BE. A Golgi fragmentation pathway in neurodegeneration. Neurobiol Dis [Internet]. 2008;29(2):221-231. Available from: https://doi.org/10.1016/j.nbd.2007.08.015

34. Valdez RC, Flock DL, Martin LJ. Endoplasmic Reticulum pathology and stress response in neurons precede programmed necrosis after neonatal hypoxia-ischemia. Int J Dev Neurosci [Internet]. 2016;48:58-70. Available from: https://doi.org/10.1016/j.ijdevneu.2015.11.007

35. Li J, Ahat E, Wang Y. Golgi Structure and Function in Health, Stress, and Diseases. Results Probl Cell Differ [Internet]. 2019;67:441-485. Available from: https://doi.org/10.1007/978-3-030-23173-6_19

36. Knott AB, Perkins G, Schwarzenbacher R. Mitochondrial fragmentation in neurodegeneration. Nat Rev Neurosci [Internet]. 2008;9(7):505-18. Available from: https://doi.org/10.1038/nrn2417

37. Devine MJ, Kittler JT. Mitochondria at the neuronal presynapse in health and disease. Nat Rev Neurosci [Internet]. 2018;19:63-80. Available from: https://doi.org/10.1038/nrn.2017.170

38. Rose S, Niyazov DM, Rossignol DA. Clinical and Molecular Characteristics of Mitochondrial Dysfunction in Autism Spectrum Disorder. Mol Diagn Ther [Internet]. 2018;22(5):571-93. Available from: https://doi.org/10.1007/s40291-018-0352-x

39. Cogliati S, Enriquez JA, Scorrano EL. Mitochondrial Cristae: Where Beauty Meets Functionality. Trends Biochem Sci [Internet]. 2016;41(3):261-73. Available from: https://doi.org/10.1016/j.tibs.2016.01.001

40. Maine EM, Hansen D, Springer D. Caenorhabditis elegans atx-2 promotes germline proliferation and the oocyte fate. Genetics [Internet]. 2004;168(2):817-30. Available from: https://doi.org/10.1534/genetics.104.029355

41. Vuksanovic DB, Snow K, Patterson MC. Spinocerebellar Ataxia Type 2 (SCA 2) in an InfantWith Extreme CAG Repeat Expansion. Am J Med Genet. 1998;79(5):383-7.

42. Kasumu AW, Hougaard C, Rode F. Selective Positive Modulator of Calcium-Activated Potassium Channels Exerts Beneficial Effects in a Mouse Model of Spinocerebellar Ataxia. Chem Biol [Internet]. 2012A;19(10):1340-53. Available from: https://doi.org/10.1016/j.chembiol.2012.07.013

43. Kasumu AW, Liang X, Egorova P. Chronic Suppression of Inositol 1,4,5-Triphosphate Receptor-Mediated Calcium Signaling in Cerebellar Purkinje Cells Alleviates Pathological Phenotype in Spinocerebellar Ataxia 2 Mice. J Neurosci [Internet]. 2012;32(37):12786-96. Available from: https://doi.org/10.1523/JNEUROSCI.1643-12.2012

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Conflict of interests

The authors declare no conflicts of interest.

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