

Letter to the Editor

Midbrain Dopaminergic Neuron-Containing Spheroids Carrying the Novel *POLG1* Variant C.2432A>G May Not Model ParkinsonismFinsterer J, MD, PhD^{*1}, Scorza Fulvio A, MD², Scorza Carla A, MD² and Fiorini Ana C, MD³¹Krankenanstalt Rudolfstiftung, Messerli Institute, Vienna, Austria²Disciplina de Neurociência, Universidade Federal de São Paulo/Escola Paulista de Medicina (UNIFESP/EPM) São Paulo, Brasil³Programa de Estudos Pós-Graduado em Fonoaudiologia, Pontifícia Universidade Católica de São Paulo (PUC-SP), Brazil; Departamento de Fonoaudiologia, Escola Paulista de Medicina/Universidade Federal de São Paulo (EPM/UNIFESP), São Paulo, Brazil

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With interest we read the article by Chumarina *et al.*, about cellular alterations in midbrain dopaminergic neuron-containing spheroids (MDNS) derived from induced pluripotent stem cells from a 28 years-old female patient carrying the novel *POLG1* variant c.2432A>G (Chumarina, M. *et al.*, 2019). The patient manifested clinically with Parkinson's disease (PD), cataract, premature ovarian failure (POF), and myopathy presenting as progressive external ophthalmoplegia (PEO) and fatigue (Chumarina, M. *et al.*, 2019). The authors concluded that use of induced pluripotent stem cells is useful for identifying altered cellular pathways possibly involved in the pathogenesis of PD (Chumarina, M. *et al.*, 2019). The study has a number of shortcomings.

The first shortcoming is that the authors did not provide the family history. We should know if the *POLG1* variant was inherited from the father or mother or if it occurred sporadically. It should be mentioned if either parent manifested with phenotypic features like those in their daughter, particularly if either parent had developed PD. Proof of pathogenicity of a novel variant requires intra-familial segregation of a variant with the phenotype over several generations. Despite POF with onset at age 28 years we should know if the index patient had children and if they carried the culprit

variant or were clinically affected. Autosomal dominant inheritance suggests that affected subjects occur in each generation.

The second shortcoming is that mtDNA sequencing was not carried out. Since *POLG1* variants frequently cause multiple mtDNA deletions or mtDNA depletion, it is crucial for interpretation of the phenotype to investigate by e.g. real-time PCR if mtDNA was secondarily affected by the mutation or not. It is also crucial that the mtDNA copy number is determined by long range PCR since *POLG1* variants were reported in association with reduced mtDNA copy number (Gui, Y. X. *et al.*, 2015). The mtDNA copy number has been even proposed as a biomarker of PD (Pyle, A. *et al.*, 2016).

The third shortcoming is that the index patient was not prospectively investigated for multisystem involvement. *POLG1* variants frequently manifest not only in the brain or muscle but also in other organs such as the eyes (cataract, optic atrophy), ears (hypacusis), endocrine organs (hypogonadism, diabetes), heart (heart block, dilated right atrium), intestines (hepatopathy, pancreatitis, cyclic vomiting), kidneys (renal tubular acidosis), and peripheral nerves (neuropathy) (table 1). In the early stages of the disease clinical manifestations

may be subclinical why it is crucial to investigate *POLG1* carriers prospectively even in the absence of overt clinical manifestations. Multisystem involvement strongly determines the disease course, prognosis, and outcome of these patients.

A fourth shortcoming is that among the impaired expression of 61 proteins in *POLG1* MDNS cells none explained the occurrence of mitochondrial myopathy in the index patient.

A fifth shortcoming is that neither serum nor cerebro-spinal fluid lactate levels were presented. Fatigue could be simply explained by lactic acidosis, which can be a manifestation of *POLG1* carriers (Inbar-Feigenberg, M. et al., 2018).

A sixth shortcoming is that the MDNS may not adequately reflect nigro-striatal neurons and may thus represent only an insufficient model of cellular dysfunction in nigro-striatal neurons of PD patients. Western blot did not show any differences between *POLG1* MDNS and controls (Chumarina, M. et al., 2019).

Table1. *POLG1* variants manifesting with PD

Variant	Age (y)	Sex	Additional features	Reference
Y955C	32	f	PEO, POF	(Pagnamenta, A. T. et al., 2006)
C2839C>T	27, 33	f, f	Neuropathy, dystonia, myopathy	(Davidzon, G. et al., 2006)
1532G>A	61	f	PEO, hearing loss, neuropathy, myopathy	(Hudson, G. et al., 2007)
Arg953Cys	33	m	PEO, neuropathy, CMP	(Gurgel-Giannetti, J. et al., 2012)
A899T	48	f	Myopathy, neuropathy	(di Poggio, M. B. et al., 2013)
R964C	58	m	Dementia, diabetes	(Hsieh, P. C. et al., 2019)
c.2432A>G	28	f	PEO, POF, myopathy, cataract	(Chumarina, M. et al., 2019)

PEO: progressive external ophthalmoplegia, **POF:** premature ovarian failure, **CMP:** cardiomyopathy

We do not agree with the notion that PD is a rare phenotypic feature of *POLG1* variants. On the contrary presence of *POLG1* variants has been repeatedly reported in PD patients (table 1) and there is even a discussion if *POLG1* polymorphisms or intronic CAG-repeat expansion may contribute to the development of PD.

Overall, this interesting study has a number of shortcomings which need to be addressed before interpreting the novel *POLG1* mutation c.2432A>G as pathogenic and causative for PD. Whether MDNS truly represent a model of dopaminergic neurons affected in PD remains speculative.

REFERENCES

- Chumarina, M., Russ, K., Azevedo, C., Heuer, A., Pihl, M., Collin, A., ... & Zini, M. (2019). Cellular alterations identified in pluripotent stem cell-derived midbrain spheroids generated from a female patient with progressive external ophthalmoplegia and parkinsonism who carries a novel variation (p. Q811R) in the *POLG1* gene. *Acta neuropathologica communications*, 7(1), 208.
- Davidzon, G., Greene, P., Mancuso, M., Klos, K. J., Ahlskog, J. E., Hirano, M., & DiMauro, S. (2006). Early-onset familial parkinsonism due to *POLG* mutations. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, 59(5), 859-862.
- di Poggio, M. B., Nesti, C., Bruno, C., Meschini, M. C., Schenone, A., & Santorelli, F. M. (2013). Dopamine-agonist responsive Parkinsonism in a patient with the SANDO syndrome caused by *POLG* mutation. *BMC medical genetics*, 14(1), 105.
- Gui, Y. X., Xu, Z. P., Lv, W., Zhao, J. J., & Hu, X. Y. (2015). Evidence for polymerase gamma, *POLG1* variation in reduced mitochondrial DNA copy number in Parkinson's disease. *Parkinsonism & related disorders*, 21(3), 282-286.
- Gurgel-Giannetti, J., Camargos, S. T., Cardoso, F., Hirano, M., & DiMauro, S. (2012). *POLG1* Arg953Cys mutation: expanded phenotype and recessive inheritance in a Brazilian family. *Muscle & nerve*, 45(3), 453.
- Hsieh, P. C., Wang, C. C., Tsai, C. L., Yeh, Y. M., Lee, Y. S., & Wu, Y. R. (2019). *POLG* R964C and *GBA* L444P mutations in familial Parkinson's disease: Case report and literature review. *Brain and Behavior*, 9(5), e01281.
- Hudson, G., Schaefer, A. M., Taylor, R. W., Tiangyou, W., Gibson, A., Venables, G., ... & Chinnery, P. F. (2007). Mutation of the Linker Region of the Polymerase γ -1 (*POLG1* Gene Associated With Progressive External Ophthalmoplegia and Parkinsonism. *Archives of neurology*, 64(4), 553-557.
- Inbar-Feigenberg, M., Blaser, S., Hawkins, C., Shannon, P., Hewson, S., & Chitayat, D. (2018). Mitochondrial *POLG* related disorder presenting prenatally with fetal cerebellar growth arrest. *Metabolic brain disease*, 33(4), 1369-1373.
- Pagnamenta, A. T., Taanman, J. W., Wilson, C. J., Anderson, N. E., Marotta, R., Duncan, A. J., ... & Rahman, S. (2006). Dominant inheritance of premature ovarian failure associated with mutant mitochondrial DNA polymerase gamma. *Human Reproduction*, 21(10), 2467-2473.
- Pyle, A., Anugraha, H., Kurzawa-Akanbi, M., Yarnall, A., Burn, D., & Hudson, G. (2016). Reduced mitochondrial DNA copy number is a biomarker of Parkinson's disease. *Neurobiology of Aging*, 38, 216-e7.